University of California Santa Cruz
Assessing SPATT in San Francisco Bay
SFEI Contract 1051

Final Report

This project involves using Solid Phase Adsorption Toxin Tracking (SPATT) to detect and quantify microcystin and other phytotoxins in San Francisco Bay, and to undertake controlled experiments using SPATT whose goal is to improve the ability to translate SPATT-derived measurements into average ambient concentrations of phytotoxins.

In **Task 1**, SPATT was deployed on San Francisco Bay cruises in a flow-through configuration and at fixed sites as part of on-going monitoring work for phycotoxins in San Francisco Bay. As discussed with SFEI, one SPATT was deployed per basin in the surface-sampling flow-through system during the monthly *Polaris* cruises. Based on adjustments to cruise schedules and cruise types, SPATT were routinely deployed on all available cruises.

In **Task 2** controlled experiments were conducted in the laboratory to better characterize partitioning of phytotoxins out of solution and into the SPATT. Experiments were designed to evaluate measurement reproducibility, and whether reproducibility can be optimized by adjusting SPATT configuration. Options include:

> a.   Controlled experiments carried out in simulated flow-through systems in which SPATT will be exposed to brackish water and seawater containing concentrations of a surrogate compound for toxins, e.g.. microcystin-RR or similar. Toxin will be quantified as a function of both dissolved concentration and exposure time. This "calibration" information will allow for more accurate back-calculations of average ambient concentrations in natural systems.

> b.   Time-series "bottle" experiments in which SPATT will be exposed in containers holding brackish water with known concentrations of a surrogate compound for toxins (e.g., microcystin-RR). SPATT will be removed at multiple time points and toxin uptake will be measured. This information will aid in characterizing the uptake kinetics of microcystin under conditions simulating deployments at a single site.

Research priories for Task 2 were identified collaboratively by Kudela and SFEI, and a project plan was developed that is feasible within the available budget.

Results—Task 1
We have processed 155 SPATT samples from USGS cruises, between October

2011 and November 2014. Additional samples (through April 2015) have also been obtained and processed, but were not included in a recent analysis as part of a separate SFEI effort. For convenience, data presented here are limited to the 155 SPATT, but we continue to process the samplers.

While we anticipated 60 SPATT per year, several of the USGS cruises were canceled or reduced in geographic range in 2013 due to ship issues. For each SPATT we have analyzed for domoic acid (DA) and microcystins LR, RR, YR, and LA. These four congeners are identified by OEHHA as the primary microcystin toxins in California, and are considered to be of equivalent toxicity. We therefore sum the congeners to report "total microcystin".

Preliminary data analysis was conducted on the SPATT and USGS underway data for presentation at several meetings:

Kudela, RM, C Mioni, M Peacock, T Schraga. San Francisco Bay acts as a reservoir and mixing bowl for both marine and freshwater toxins. Coastal and Estuarine Research Federation, 3-7 November 2013, San Diego, CA.
Kudela, RM, C Mioni, M Peacock, T Schraga. San Francisco Bay acts as a reservoir and mixing bowl for both marine and freshwater toxins. Eastern Pacific Oceans Conference, Fallen Leaf Lake, California, September 17-19, 2013.
Kudela, R, Peacock, M, Schraga, T, Senn D. 2014. Does San Francisco Bay have a harmful algal bloom problem? 2014 Bay-Delta Science Conference, 28-30 October 2014, Sacramento, CA.

Those presentations are used as the basis for this interim report.

Between 2011-2014, 25 Full Bay and 28 South Bay cruises were analyzed. From those samples, 71.5% were positive for microcystins and 96.5% were positive for domoic acid (Figure 1). Concentrations ranged from 0-400 ng/g domoic acid, and 0-25 ng/g microcystins (Figure 2). Peaks in both toxins were coincident in time, and appear to be related to river flow. Moderate river flow is associated with the highest toxin concentrations. Spatially, toxins were fairly uniformly distributed throughout the four basins (Figure 3). During some periods there was clear separation based on temperature-salinity (T-S) properties, with domoic acid associated with "marine" waters and microcystins associated with "fresh" waters. However at other times toxins were distributed without a clear pattern through the Bay (Figures 4, 5).

The range of toxin concentrations, range of environmental parameters, and length of the time-series (3+ full years of data) make this dataset conducive to statistical modeling to identify relationships between toxins and environmental drivers or correlates (see Recommendations below).

Results—Task 2
We have conducted several "bottle" experiments to evaluate SPATT adsorption under representative conditions. In particular, we recently expanded the SPATT methodology to include anatoxin-a. This is a potent neurotoxin also known as "sudden death factor". While there are no reports of anatoxin-a for San Francisco

Bay, we have routinely seen elevated levels in the Eel River, and occasionally get positive hits in nearby Pinto Lake. We recently concluded a laboratory calibration for anatoxin-a, looking at adsorption and recovery efficiency, effect of different source waters, and effect of temperature on adsorption.

We can now quantify SPATT (using HP20 resin) characteristics for domoic acid, microcystins, and anatoxin-a. Excitingly, we can use a single extraction method to analyze all three toxins from the same SPATT. We can also analyze for okadaic acid (Diarrhetic Shellfish Poisoning), extending our capability to 4 toxins that cover the majority of compounds expected in San Francisco Bay.

Because SPATT and grab samples (or indicator organisms) are fundamentally different measurement methods, we do not recommend a direct calibration factor between the various toxin detection methods. Rather, we provide ranges of SPATT concentrations that correlate to management action levels. For example, OEHHA recommends an alert or action level of 0.8 ppb total microcystins. Based on the large comparative dataset, this would be equivalent to a threshold concentraton of ~1-4 ng/g for SPATT (see below). Based on that criteria, San Francisco Bay appears to approach this alert level seasonally (Figure 2).

Additional Analyses
We requested an extension to the contract to more fully characterize SPATT. We proposed to complete the following:

• SPATT deployment/analysis through 2014, providing a full 3-year record
• Finish characterization of toxin uptake in a simulated flow-through system
• Prepare a peer-reviewed publication describing the presence of toxins in San Francisco Bay

We further recommended the following. These five recommendations are beyond the scope of the current contract, but could be implemented within a 12-18 month contract at a similar cost to this contract.

1) Continue SPATT deployment beyond the scope of this contract;
2) Analyze matched filter samples from the USGS cruises for particulate toxins, to further calibrate the SPATT data; this could also be compared to an existing dataset of HPLC pigments and microscopy samples;
3) Analyze archived mussel tissue provided by the RMP as a pilot dataset, to determine whether additional sample analysis is warranted. This would directly link toxins to trophic accumulation.
4) Develop a method for saxitoxins. This is the only toxin group that we know is in SFB that is not currently included in our analysis. It requires some personnel time to set up the method, and supplies costs.
5) Analyze archived SPATT for anatoxin-a and okadaic acid.

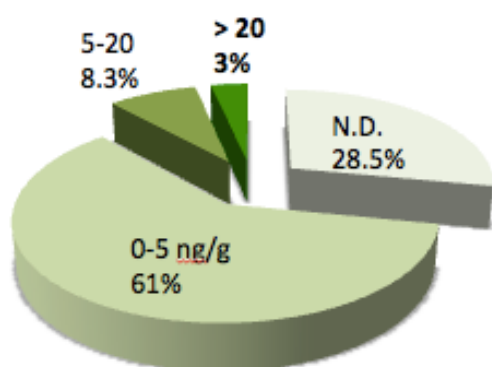## Results from the Contract Extension

Following this document, we provide a separate write-up for the laboratory characterization (the second bullet from the proposed contract extension). Analysis of SPATT through 2014 was also completed, and an initial write-up with peer-review is ongoing as part of the following report and manuscript:

Sutula, Martha , Raphael Kudela, James Hagy, Gry Mine Berg, Suzanne Bricker, James E. Cloern, Richard Dugdale, Lawrence W. Harding, Jr., and David Senn. 2015 (in prep.). Scientific Basis for Assessment of Nutrient Impacts on San Francisco Bay.
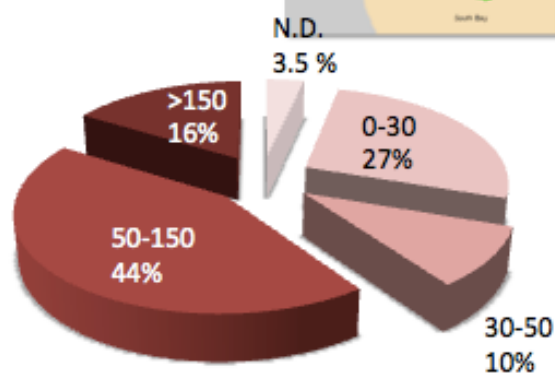
2011-2014: USGS Deployment of SPATT

October 2011 – July 2014
- 25 Full-Bay cruises
- 28 South Bay cruises

Microcystins

- > 20: 3%
- 5-20: 8.3%
- N.D.: 28.5%
- 0-5 ng/g: 61%

Domoic Acid

- N.D.: 3.5 %
- >150: 16%
- 0-30: 27%
- 50-150: 44%
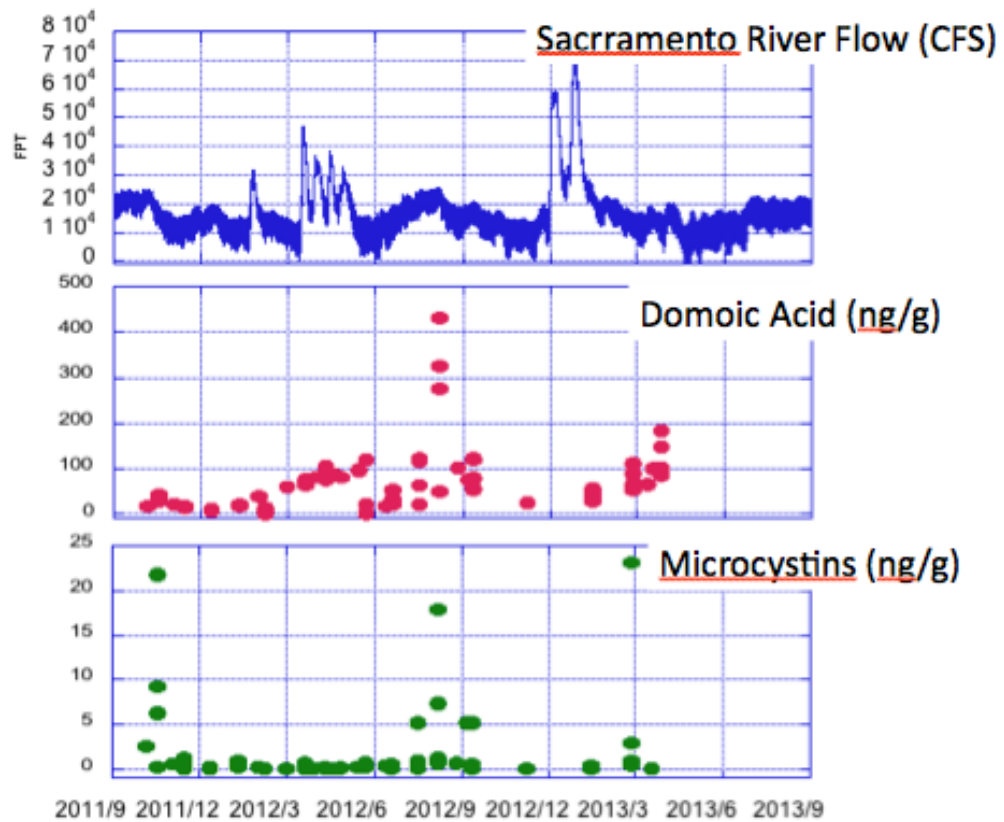- 30-50: 10%

Figure 1. Summary results from the USGS cruises.

Figure 2. Toxin data shown as a time-series, with river flow (top). Toxins are generally associated with moderate flow in the autumn. The two peaks in autumn 2011 and summer 2012 are shown in more detail in Figures 4-5.
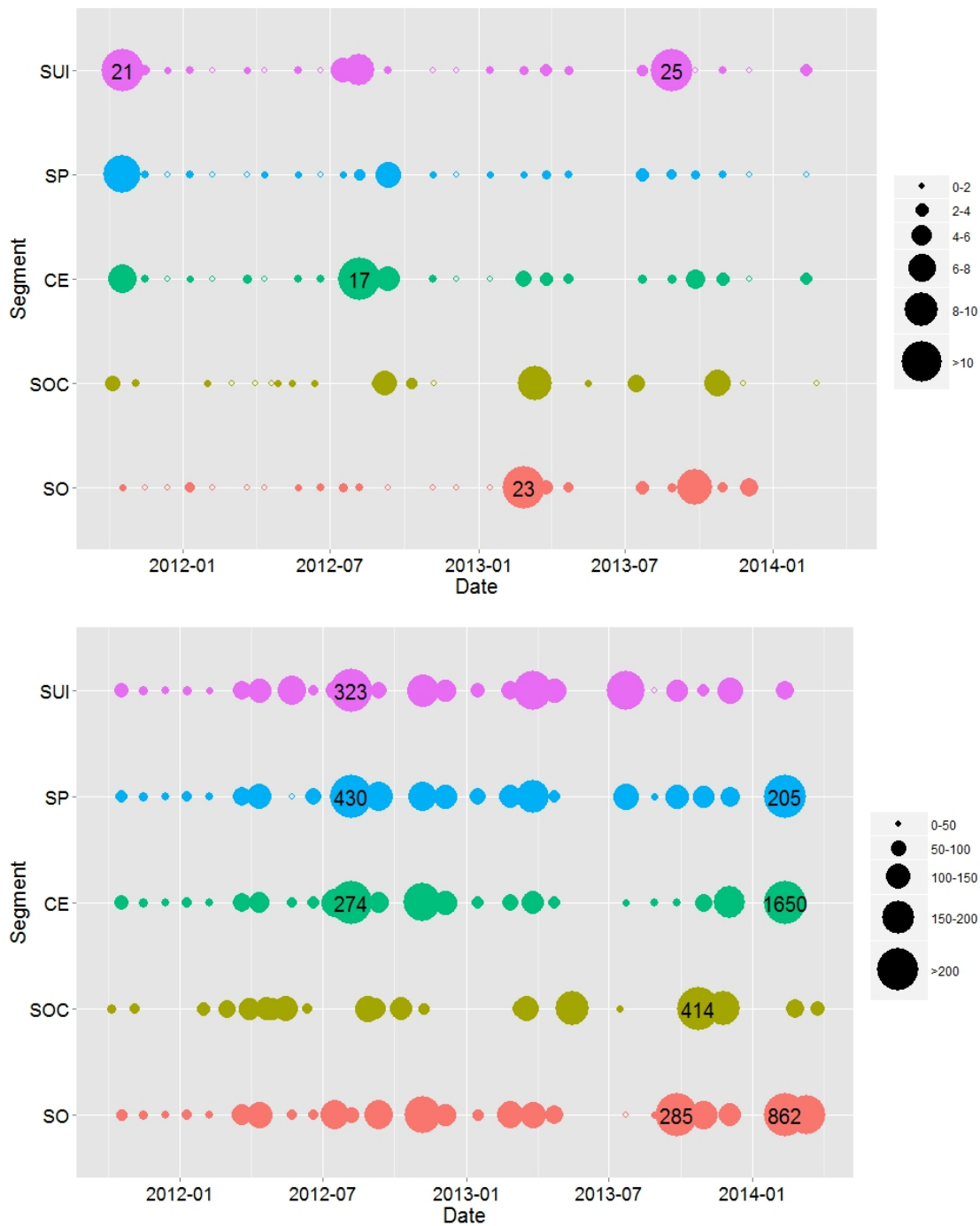
Figure 3. All of the SPATT data shown as concentration (larger circle equals more toxin). Note that microcystins are easily detectable, but fairly low. DA values are fairly high. Letter codes refer to subembayment: SO=South Bay, SOC=South Central, CE=Central, SP=San Pablo, SUI=Suisun.

Figure 4. Toxin data plotted in T-S space. For this period, microcystins are clearly coming from the Delta, and spreading into the rest of the Bay, while DA is coming from Central Bay and spreading into the rest of the Bay, suggesting that sometimes, it's simply conservative mixing that is moving the toxins around.

SPATT concentrations plotted in Temperature-Salinity space

Bubble size = toxin concentration; color corresponds to Bay regions

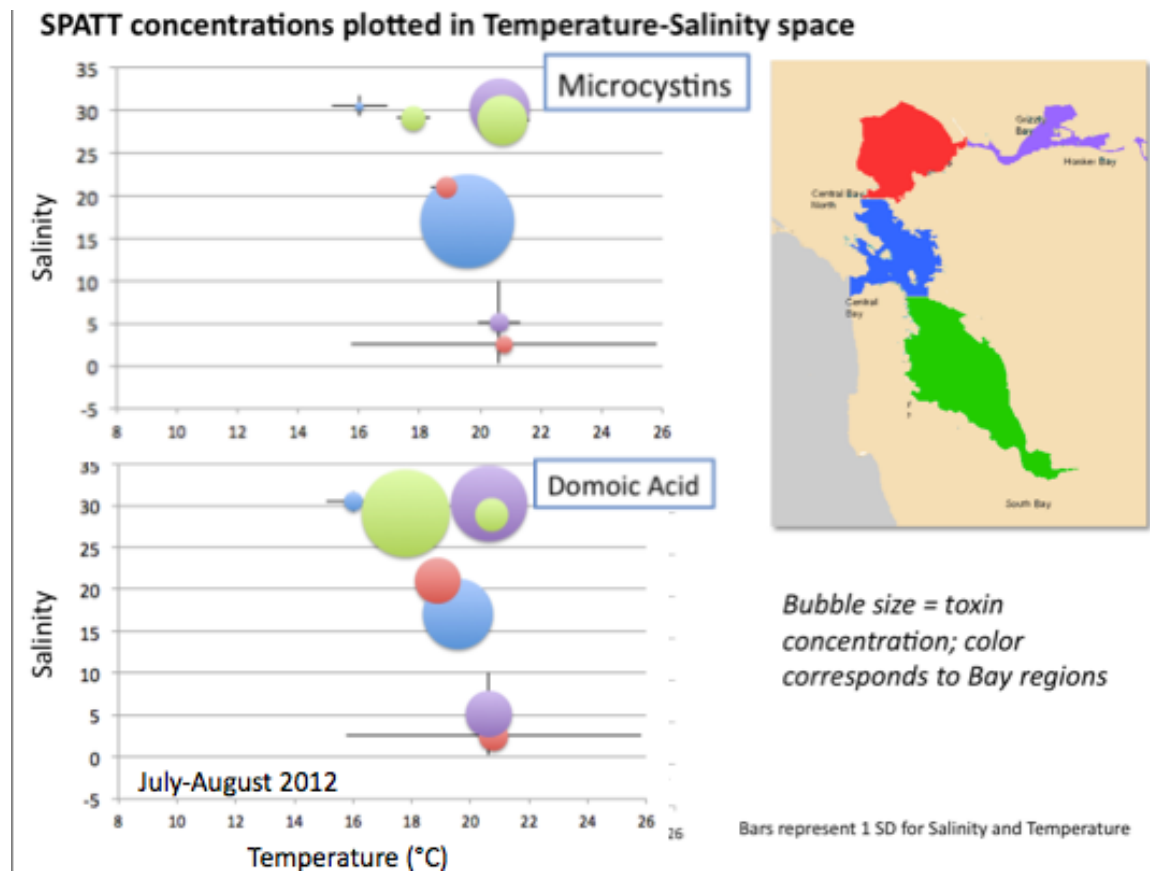*Bars represent 1 SD for Salinity and Temperature*

Figure 5. Another example, from July-August 2012. It is not as clear how the geographic patterns relate to environmental forcing. Highest microcystins are in Central Bay, with moderate levels in South Bay and the Delta. There is evidence (not shown) that microcystins are coming in from a separate South Bay source, possibly the sloughs and salt ponds. The DA is highest in South Bay, and pretty high in the Delta, suggesting transport of cells that eventually release toxin.

Results from Contract Extension
Calibration of SPATT—Background

A primary objective of this project was to intercalibrate SPATT toxin data for microcystins and domoic acid such that data from the USGS underway mapping aboard the R/V *Polaris* can be qualitatively related to regulatory limits. OEHHA recommended 0.8 ppb for the sum of total (particulate and dissolved) microcystin LR, RR, YR, and LA. There are no formal guidelines for domoic acid, but regulatory limits for fish and shellfish is 20 ppm in tissue.

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 domoic acid are not equivalent to toxin levels in 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.
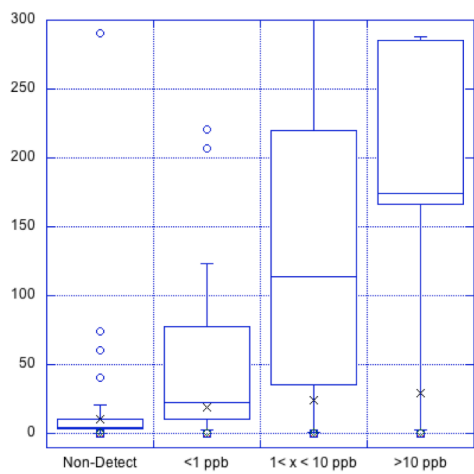

Figure 1. SPATT data from Pinto Lake, showing the correspondence between grab sample bins and SPATT values.

An initial attempt to provide an intercomparison used environmental data from long time-series at the Santa Cruz Municipal Wharf (Lane et al. 2010) and from Pinto Lake, California (Kudela 2011). For both of those programs SPATT, using the HP20 resin, are 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 an 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 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 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 corresponding to mussel tissue domoic acid concentrations from matching mussel samples (SPATT were deployed weekly; mussels 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

Calibration of R/V *Polaris* Underway Measurements

In order to translate the general characteristics of HP20 SPATT, a simulation was set up in the laboratory to mimic conditions on the R/V *Polaris* cruises. 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 (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, so 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 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. 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, replicate (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. Upper photo shows the carboy, receiving container, and pump. Lower photo shows SPATT (in 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.
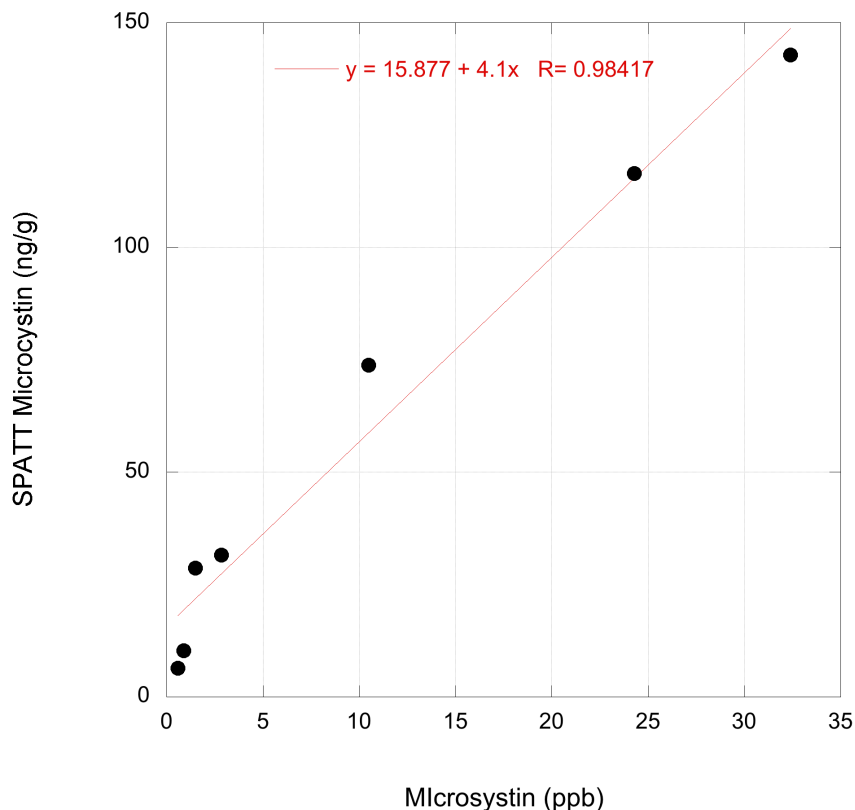

Figure 3. SPATT versus ambient water concentration for microcystins for SPATT exposed to constant concentration of toxin for 15 minutes. When forced to a zero-intercept, the calibration factor is 4.97x.

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.
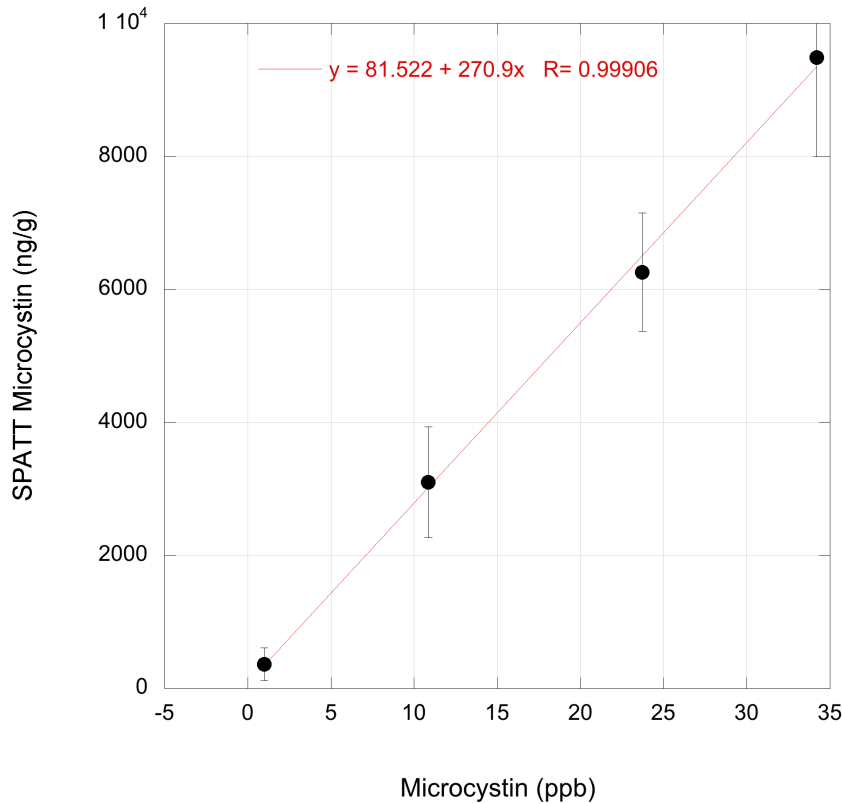

Figure 4. SPATT versus ambient water concentration for microcystins with SPATT exposed to a constant concentration for >1 hour.

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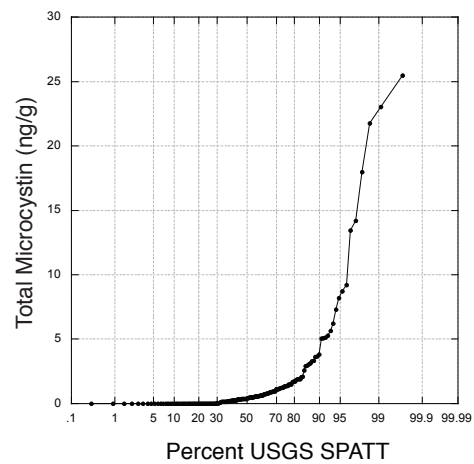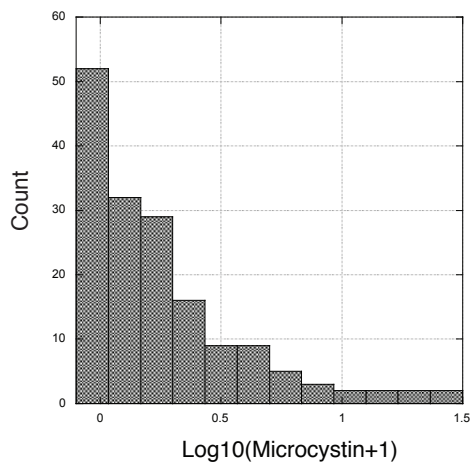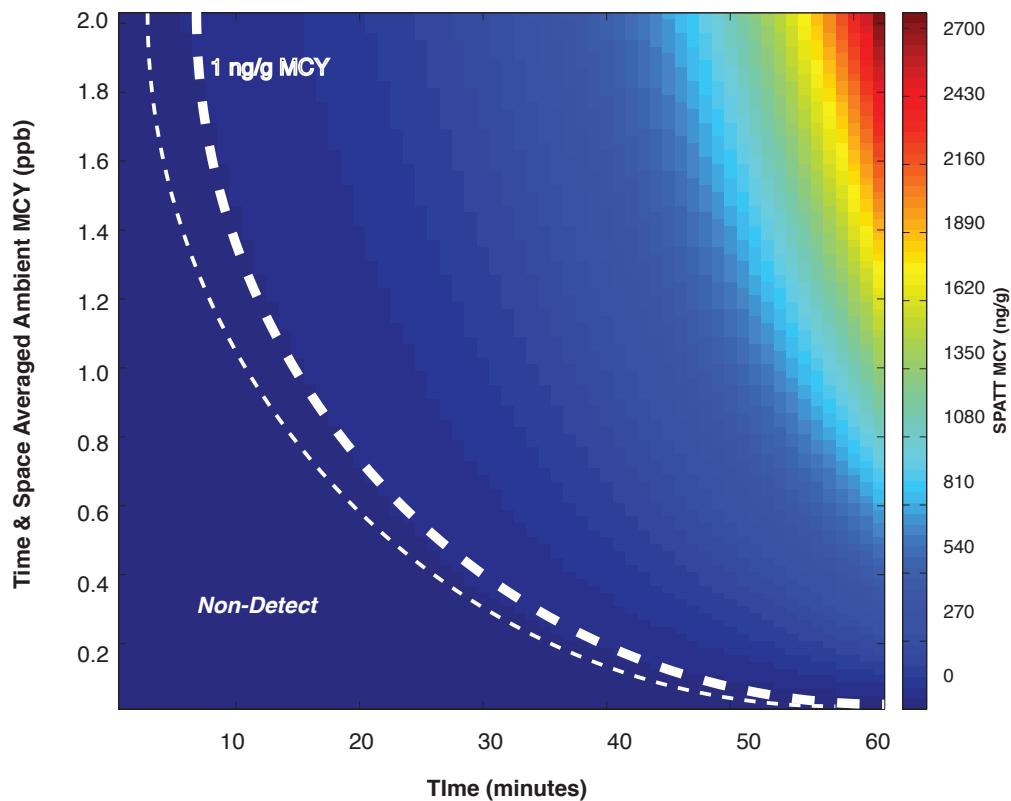
Figure 5. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~1 hour. The lower panels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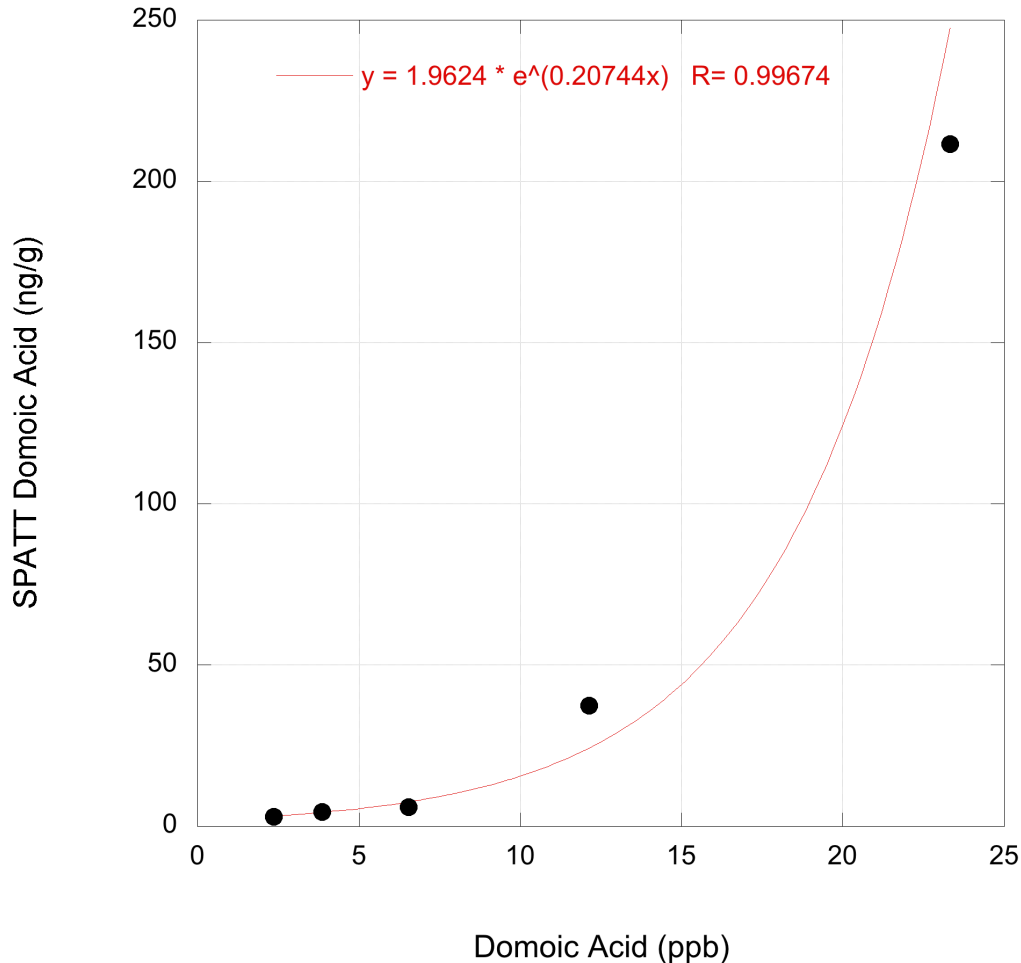

Figure 6. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for 15 minutes.

Domoic Acid, > 20 hour exposure: Testing of SPATT showed that domoic acid continues to be adsorbed for up to 24 hours, 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 assume using a linear relationship.
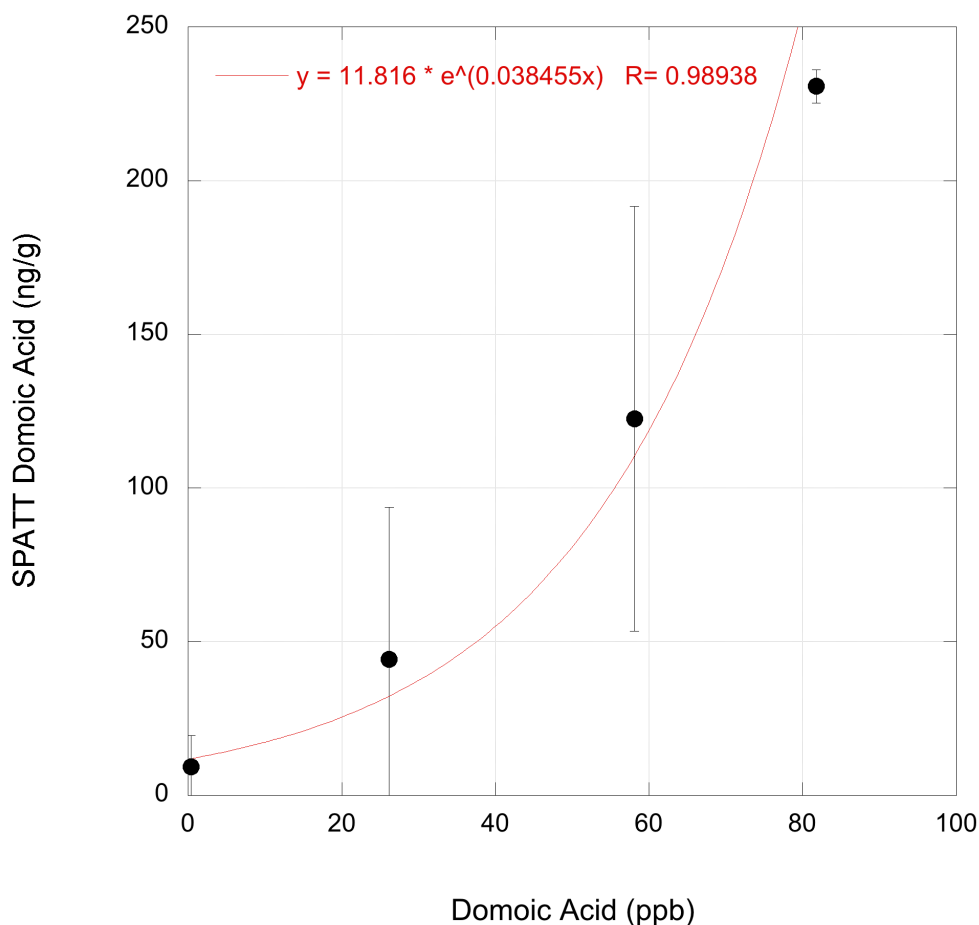
Figure 7. SPATT versus ambient water concentration for domoic acid for SPATT exposed to constant concentration of toxin for >20 hours.

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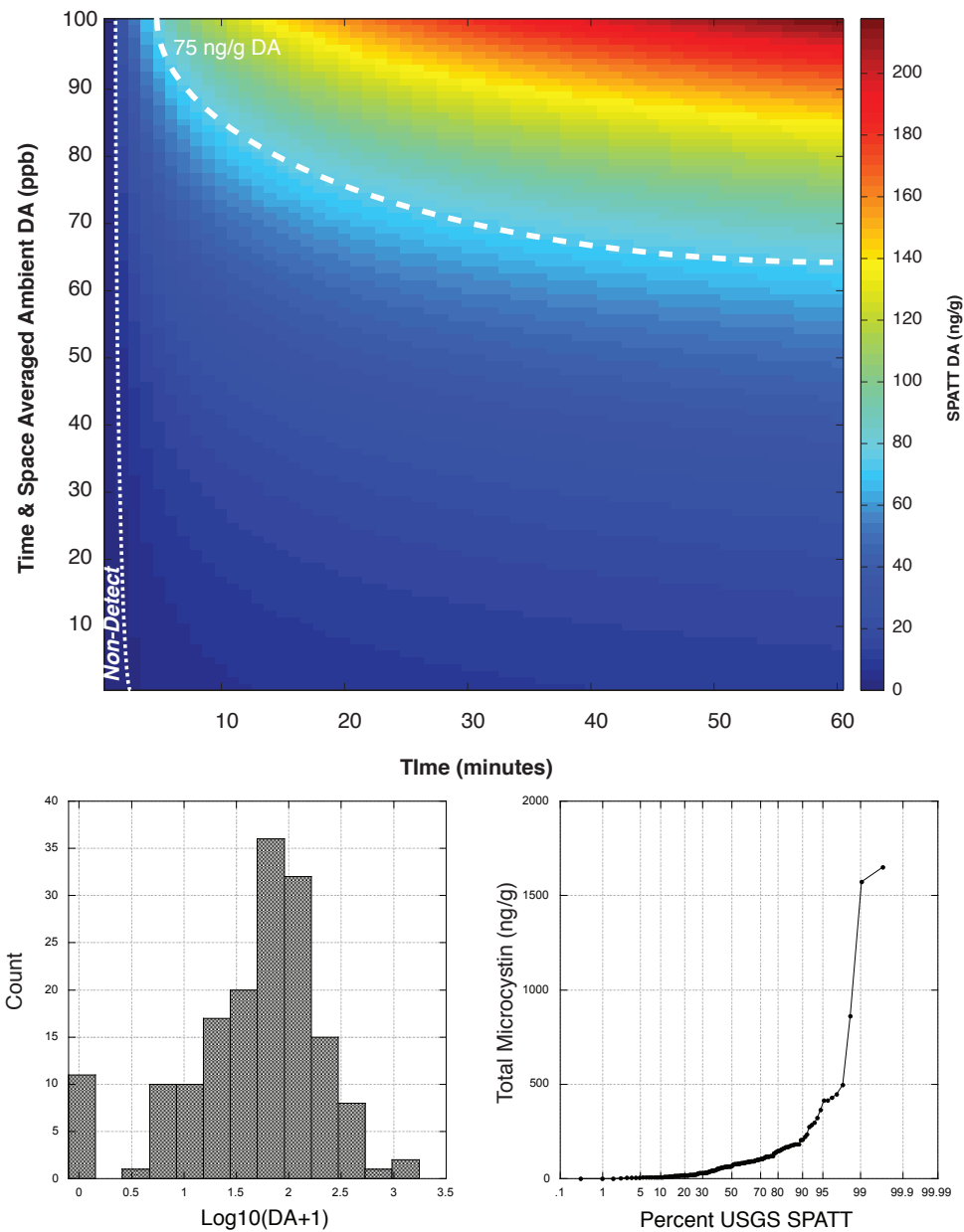
Figure 8. The top graph shows the SPATT concentrations that would be measured as a function of exposure time versus concentration. Note that toxin levels would increase/decrease in response to exposure (x-axis) to water with higher/lower concentration, with an equilibrium time of ~several days. The lower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

Calibration of SPATT—Comparison to Mussels

Mussel samples were obtained from the RMP monitoring program for 2012 and 2014. This provides a direct comparison between a regulatory measurement (tissue samples) and SPATT from approximately the same time period and location, keeping in mind that the SPATT are deployed in surface water on a subembayment scale for a few hours, while mussels are deployed at depth for ~6 months.
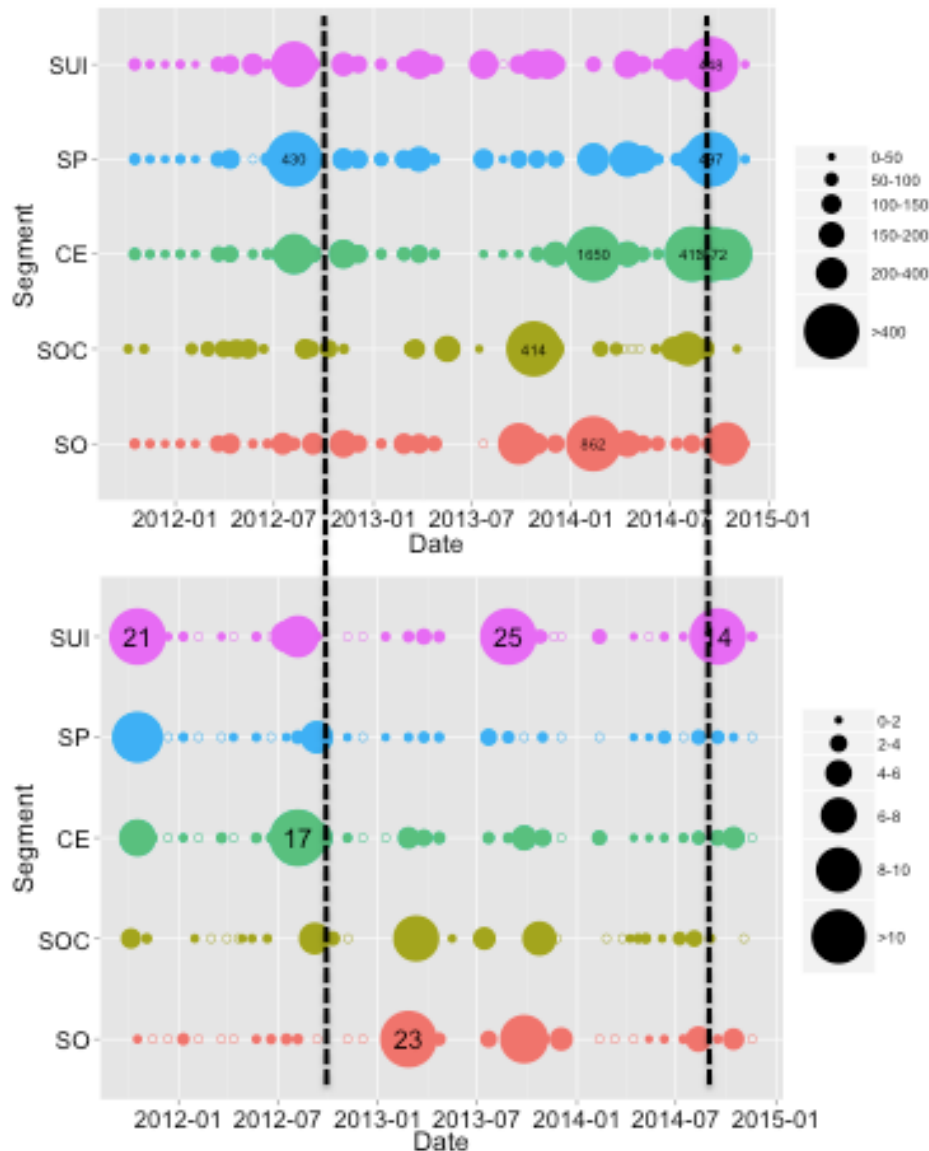

Figure 9. SPATT time-series, with bivalve retrieval dates overlayed as dashed lines. Note that bivalves were retrieved shortly after widespread toxin throughout the Bay for both DA and microcystins.

Figure 9 shows the SPATT time-series for microcystins and DA, with the mussel collection. Note that toxin was detected in mussels immediately following periods when SPATT indicated widespread presence within the Bay. For the mussel samples, 100% of sites had detectable domoic acid, while 82% (2012) and 100% (2014) of mussels had detectable microcystins. Of the two, the microcystins were closer to regulatory closure, with a maximum value of ~22 µg/kg (WHO guidelines recommend closure at 24 µg/kg). Comparison of SPATT with the mussel data suggest that a microcystin level of 10-20 ng/g SPATT would be too conservative, so more recent recommmendations have lowered this to 1 ng/g. Similarly, presumably because of the non-linearity in uptake, DA values of 30-50 ng/g are probably too conservative, and the new recommended value is 75 ng/g (these values are reported in Sutula et al., in prep; "Scientific Basis for Assessment of Nutrient Impacts on San Francisco Bay").

Calibration of SPATT--Recommendations

Based on this initial pilot study of field-deployed SPATT and laboratory calibration, it seems clear that the SPATT time-series should be continued as part of the USGS cruises. Discussions with USGS and SFEI have explored the possibility of further dividing the Bay into subembayments consistent with the analysis performed by Sutula et al. (in prep.). This would primarily mean adding a Lower South Bay SPATT sampler, and separating Central Bay and North Central Bay. It is also recommended that, if possible, additional mussel samples be collected since this is the most unambiguous comparison between SPATT and ecosystem impairment. As part of separate SFEI funding, analysis is also underway to compare discrete filter samples with SPATT, but this will be subject to sampling variability (in previous comparisons, >50% of grab samples were negative while SPATT was positive) and to issue with limits of detection using filters due (primarily) to the heavy sediment load encountered when filtering whole water. It would also be useful to conduct a statistical analysis of SPATT relative to environmental conditions, to identify likely drivers of variability. Finally, additional laboratory testing of SPATT adsorption/desorption (for example, in response to salinity) could be carried out.

Ranking these recommendations by feasibility, cost, and impact, the following is proposed (from highest to lowest), with the recommendation followed by comments [in brackets]:

1) Continue SPATT time-series.

[SPATT is ongoing, primary limitation is availability of funds for both deployment and analysis of the data].

2) Collect additional mussel (or other invertebrate) samples for toxin analysis compared to SPATT. Ideally, deploy SPATT co-located with mussels.

[Feasible, but RMP currently conducts experiments every 2 years. So costs increase considerably if more frequent sampling is desired].

3) Add Lower South Bay and North Central Bay to the existing SPATT time-series.

[Minimal additional effort; would require permission from USGS, and would increase current costs by about 25%].

4) Collect/analyze discrete plankton samples for toxins to compare with SPATT.

[This is underway as part of separate funding; it's not clear that it will provide a direct intercalibration, given the past issues with comparing grab samples and SPATT].

5) Conduct retrospective analysis of SPATT versus environmental conditions to identify drivers of variability.

[This is probably a high priority, but the longer the time-series, the more valuable the analysis; analyzing now would primarily capture the drought period. Consider waiting until the drought ends, or anticipate analyzing again in the future. This could be a task for SFEI via the funded project for Blakely in 2015-16].

6) Conduct additional laboratory intercalibration.

[This could be done, but given the data already presented and the recent publications on SPATT, the chemistry is reasonably well-constrained. It would be a low priority compared to intercalibration with field samples].

Final Recommendation for interpreting SPATT: as documented in the Sutula et al. (in prep.) document, current recommendations based on statistical analysis, comparison with other field sites, and comparison with limited mussel samples is to consider "elevated" toxin concentrations equivalent to 1 ng/g total microcystins or 75 ng/g domoic acid for SPATT deployed by subembayment in San Francisco Bay. Values should be considered as "ranges" rather than absolute concentrations. For example, reasonable ranges, based on these updated thresholds, would be <1, 1-10, >10 for microcystins (no threat, moderate threat, high threat), and <50, 50-150, >150 for domoic acid. These ranges could be improved with additional mussel sampling, using logistic regression to define probability ranges (e.g. Lane et al. 2009; Anderson et al. 2011).

References

Anderson, CR, RM Kudela, C Benitez-Nelson, E Sekula-Wood, CT Burrell, Y Chao, G Langlois, J Goodman, and DA Siegel. 2011. Detecting toxic diatom blooms from ocean color and a regional ocean model, *Geophysical Research Letters* 38, L04603, doi:10.1029/2010GL045858.

Gibble, CM, RM Kudela. 2014. Detection of persistent microcystin toxins at the land-sea interface in Monterey Bay, California. Harmful Algae, 39: 146-153. doi:10.1016/j.hal.2014.07.004.

Kudela, RM. 2011. Characterization and Deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and salt water. *Harmful Algae*, 11: 117-125, doi: 10.1016/j.hal.2011.08.006.

Lane, JQ, GW Langlois, and RM Kudela. 2012. Update on the application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of domoic acid. Proceedings of the 14th International Conference on Harmful Algae, Intergovernmental Oceanographic Commission of UNESCO.

Lane, JQ, P Raimondi, and RM Kudela. 2009. The development of a logistic regression model for the prediction of toxigenic *Pseudo-nitzschia* blooms in Monterey Bay, California. *Marine Ecology Progress Series*, 383: 37-51.

Lane, JQ, CM Roddam, GW Langlois, and RM Kudela 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of domoic acid and saxitoxin in coastal California, *Limnology and Oceanography Methods,* 8:645-660.

Lin, F., G. Sun, J. Qiu, Q. Ma, P. Hess, A. Li. 2014. Effect of seawater salinity on pore-size distribution on a poly(styrene)-based resin and its adsorption of diarrhetic shellfish toxins. J. Chromatography A, 1373: 1-8.

Zendong, Z., C. Herrenknecht, E. Abadie, C. Brissard, C. Tixier, F. Mondeguer, V. Séchet, Z. Amzil, P. Hess. 2014. Extended evaluation of polymeric and lipophilic sorbents for passive sampling of marine toxins. Toxicon, 91:57-68.

Zhao, H., et al. 2013. Mechanism and application of solid phase adsorption toxin tracking for monitoring microcystins. *Journal of Chromatography A* 1300: 159-164.