Title

Solid Phase Absorption Toxin Tracking (SPATT) and Nutrient Loads: Testing a New Tool for Algal Toxin Monitoring in Fresh, Brackish and Coastal Ecosystems

Author

Kudela, Raphael

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Solid Phase Absorption Toxin Tracking (SPATT) and Nutrient Loads: Testing a New Tool for Algal Toxin Monitoring in Fresh, Brackish and Coastal Ecosystems

Raphael Kudela
UC Santa Cruz
kudela@ucsc.edu
831-459-3290

Project Hypotheses
We are interested in testing a new methodology called Solid Phase Absorption Toxin Tracking (SPATT), which is a man-made resin that passively captures algal toxins in water; it can be thought of as a man-made, passive, sentinel "mussel". This technology was developed in New Zealand (MacKenzie et al. 2004), and is in limited use in the UK (Turrell et al., 2007) and Australia (Takahashi et al., 2007), but has not yet been tested in the US. Our hypothesis was that this resin, deployed using the same methodology as described by others (outside the US), would passively capture domoic acid, providing an integrated time-series of toxin levels in the coastal ocean.

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 with traditional water quality sampling to characterize these known and emerging toxins. Specific objectives include:

1) Characterize SPATT in the laboratory with domoic acid, saxitoxin, yessotoxin, and microcystins using a range of salinities in artificial seawater and natural samples, to ensure that SPATT resin is at least semi-quantitative under typical conditions.

2) Deploy SPATT as part of ongoing monitoring programs at the Santa Cruz Municipal Wharf and Elkhorn Slough to field-test the toxin capture and to collect data on the linkages between eutrophication, environmental conditions, and toxin concentrations.

3) Test the deployment of an ISCO water sampler in conjunction with the SPATT resin to obtain high temporal resolution sampling of nutrients and phytoplankton abundance in conjunction with metered flow through the SPATT resin (using the ISCO sampler).

Briefly describe project methodology
Our approach can be separated into three components. First, we will evaluate SPATT in the laboratory to ensure that it works in California waters and is quantitative. Second, we will incorporate SPATT into three existing monitoring programs. Third, data will be analyzed to identify correlations between ecological conditions, toxin presence, and wildlife impacts.

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 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 saxitoxin, DA, YTX, and microcystins). Toxin analysis will be conducted using ELISA plates for DA, microcystins, and saxitoxins, and using a new, state of the art Agilent LC-MS system we are setting up specifically for analysis of algal toxins. For brevity, we will not provide all of the details, but we will follow experimental protocols similar to the recent inter-comparison we carried out for a new DA ELISA method (Litaker et al., submitted). Our group has extensive experience developing analytical methods for toxin analyses, and we have previously identified these same toxins from several new regions globally (Armstrong and Kudela, 2006; Fawcett et al., 2007; Howard et al., submitted; Litaker et al., submitted). We have budgeted for two ELISA plates, one for microcystins and one for saxitoxin (Envirologix, Inc.). We have access to ELISA plates for DA at no cost to Sea Grant. We have budgeted for supplies necessary to set up the toxin analyses on the LC-MS system, primarily for certified standards.

Field Sampling: The crux of this proposal is the field validation of SPATT, and simultaneous collection of nutrient and wildlife data. Fortunately, we are already involved with three monitoring programs that we can leverage, allowing us to test new methodologies without having to reproduce an entire field sampling effort. First, as part of Cal-PReEMPT, we sample weekly from the Santa Cruz Wharf for nutrients, chlorophyll, temperature, salinity, numerical abundance of HAB organisms (using microscopy and molecular probes), Jellett Rapid Extraction kits with sentinel mussels, domoic acid, and saxitoxin. This has been ongoing since 2003 and will continue for the duration of this project. Second, as part of the Central and Northern California Ocean Observing System (formerly the Center for Integrated Marine Technology) we have added indicator pathogen bacteria to the SC Wharf sampling site, and collect the same data 6 times per year at approximately 10 stations in Monterey Bay (see http://cimt.ucsc.edu). Third, as part of a CDFG project, nutrient, pathogen, and pollution loads are being assessed at a variety of coastal Monterey sites (see Figure 1). Jenny Lane (requested Sea Grant Trainee) has been coordinating with that group for the last year to add urea, toxin, and phytoplankton sampling to their program. All analyses are conducted following JGOFS, EPA, and/or state guidelines.

Describe progress and accomplishments toward meeting goals and objectives
We successfully completed Project Objective 1, testing the SPATT resins for absorption efficiency of various toxins. We ended up focusing primarily on domoic acid (see "Project Modifications" below) since this is the primary issue encounted in California. During the 12 month project, there were never any significant levels of yessotoxin or saxitoxin in the Monterey Bay; we therefore conducted trials with standards, but were not able to field-test for those toxins.

We also deployed the SPATT resins weekly (each sample is an integrated measurement of 1 week) at the Santa Cruz Wharf. Because of issues with the resin, we chose not deploy resins at Elkhorn Slough, but we did deploy resins in Pinto Lake, CA, during a Microcystis bloom, and successfully qualitatively

extracted microcystins from the resin (at the time of the deployment, we had qualitative, but not quantitative, toxin kits for microcystin; future sampling should be able to quantify toxin load). More details of our field sampling are provided below.

Field deployments of nitex bags containing SP700 and HP20 resins began on July 15, 2008 at the Santa Cruz Municipal Wharf. These deployments have continued on roughly a weekly basis since that date. Each bag is clipped into its own embroidery hoop, which remains fastened by zip tie to a weighted rope. The embroidery hoops are suspended at a level roughly even to the level at which biotoxin monitoring mussels are suspended nearby. The deployment of our SPATT bags alongside these mussels allows for the direct comparison between toxin detection by SPATT and that by sentinel shellfish, which are continusouly deployed and analyzed along the California coastline by the California Department of Public Health (CDPH) and its volunteers. In addition, this site is near to where water samples are collected on a weekly basis for phycotoxin analysis and for the quantification of toxic phytoplankton species.

For Objective 3, an automated sampler, the ISCO 3700, was purchased to assess its potential use with the SPATT resins we expected to identify as the most promising for DA-monitoring purposes. This automated water sampler can be deployed with the SPATT resins in one of two possible ways: (1) SPATT bags are placed in 400 mL glass bottles into which water samples are automatically dispensed, and (2) the ISCO is reconfigured so that it can accommodate a resin column through which water is pumped continuously or near-continuously. The potential for the latter configuration is limited initially by the identification of a resin with adsorption kinetics sufficiently fast, efficient, and/or well described. In our results thus far, the SP207SS resin has demonstrated the most promising adsorption kinetics for this application, but its true applicability remains an open question requiring direct interrogation of adsorption efficiencies within this specific and intended configuration.

We have had a meeting with Kinnetics Laboratories, a local company that has configured other ISCO 3700 for use with resins and resin columns. While Kinnetics Laboratories can configure the ISCO automated sampler for use with a column, the column they deploy is supplied by AXYS Technologies (supplied as ready-to-deploy and returned to AXYS for analysis). We have contacted AXYS and expect to purchase a stainless steel column such as those regularly deployed to Kinnetics Laboratories for use with the ISCO automated samplers. Once this column is received, and the necessary configurations to the ISCO 3700 are complete, trials with the resins (likely the SP207SS and SP207) will begin.

Project modifications

We ran into significant issues with the chemistry of the SPATT resins, and identified several problems not previously reported by others. The main issue is that the SPATT resin, while it does indeed absorb domoic acid and other toxins, does not readily release the toxins (necessary for quantification) following the guidelines published elsewhere. We therefore spent considerable time optimizing absorption and recovery in the laboratory, primarily for domoic acid. Below we detail our extensive testing.

Creation of the SPATT bag design

The resins used for SPATT are supplied as free resin beads, and their deployment in the field requires some form of containment. Some designs that have been developed and implemented successfully elsewhere include:

- Sandwiching the resin between two sheets of nylon mesh and securing with an embroidery hoop.
- Sewing the resin into nylon mesh bags.
- Enclosing the resin in nylon mesh with use of a zip-tie.
- While all of these designs have proven useful and appropriate elsewhere, we elected to develop and trial a new design. In this new design, the resin was sealed into nylon mesh bags using a plastic bag sealer.
- Advantages we encountered from use of this design include the following:
- The establishment of a reliable seal: secure retention of the resin within the mesh bag
- The pliability of the bags allows the resin to be to manipulated during extraction without the risk resin bead loss.
- This method does not require any sewing equipment or sewing skills.
- This method requires only the availability of a plastic-bag sealer, a low-cost device that is generally available worldwide.

Bags of the resins were created as follows:
1. Swaths of 100 •m Nitex Bolting Cloth (Part# 24-C34) were purchased from Wildlife Supply Company (www.wildco.com).
2. The nitex cloth was sealed with a plastic bag sealer on 3 sides so as to form a 55 mm x 55 mm bag once sealed shut.
3. The nitex bag was filled with 3g (dry weight) of resin.
4. The open edge of the nitex bag was sealed shut with a plastic bag sealer.
5. The resin bags were 'activated': soaked in 100% MeOH for ~72 hours, followed by rinsing and 10 minute sonication in DI-water.
6. Activated resin bags were stored in DI-water at 4°C until use.
In field deployments, the bags were secured to a weighted rope using embroidery hoop and zip tie.

Laboratory Trials

Previous SPATT laboratory and fieldwork had focused on the use of DIAION HP20, a polyaromatic adsorbent resin (styrene-divinylbenzene matrix). This resin, however, was designed for use with hydrophobic compounds and most of its application in SPATT had been with lipophilic toxins. Domoic acid (DA) is a hydrophilic molecule, suggesting that the HP20 resin may have reduced applicability in adsorption of this toxin and in its detection in the field.

As a starting point, we elected to trial both the HP20 resin and a new resin recently identified as useful with DA through work by Liz Turrell (and colleagues) within the Biotoxins and Applied Microbiology group of the Fisheries Research Services (FRS) Marine Laboratory in Aberdeen, UK. Although no results have been published or formally presented, this polystyrene-based resin, SEPABEADS SP700, was reported to have demonstrated good applicability towards DA in terms of its adsorption and recovery efficiencies, and was suggested by the FRS-UK group as an excellent candidate for our trials.

Trial 1: DA-fortified DI-water; analysis by ELISA

In this preliminary trial, a single bag of each resin type (HP20 and SP700) was soaked in DA-fortified DI-water and an adsorption series was collected. Extraction of the resin bags was by a modified protocol that had been obtained from the FRS-UK group. This initial trial was designed strictly as a 'first run', to familiarize with the adsorption and extraction process and with the general behavior of the resin.

Trial 2: DA-fortified DI-water; analysis by LC-MS

Trial two was a more formal execution of the steps taken in trial one. Each resin type (HP20 and SP700) was interrogated in triplicate, and a triplicate control sample was included in the experimental design. Analysis of all samples was by LC-MS; the use of DA-fortified DI-water (as opposed to seawater, for example), allowed us to observe the adsorption and extraction behavior without having to run the samples through SAX treatments (for salt removal), which introduces an additional analytical step and therefore an additional potential source of error within the final result.

This trial indicated that the SP700 resin was more efficient than the HP20 resin in terms of adsorption; the SP700 resin adsorbed 100% of the DA within ~48 hours, while the HP20 resin appeared to plateau at ~60% adsorption over the experimental time series.

In extraction, this trial revealed an important aspect of the HP20 resin; the HP20 displayed generally 'leakier' behavior. Just prior to extraction, field resins would be subjected to a DI-water rinse; it was therefore important to assess whether this pre-extraction rinse step had extractive effects in its own right. With the SP700 resin, no DA was detected in the DI-water rinse. With the HP20 resin, a significant amount of DA was detected in the DI-water rinse. The amount detected in the DI-water rinse exceeded the amount of DA that might have been carried over into the DI-water rinse as a result of incomplete adsorption.

With the HP20 resin, extraction was more complete overall (~45%). Extraction efficiency for the SP700 resin was minimal, and much less than what had been anticipated based on our conversations with the FRS-UK group (observed extraction efficiency of 80-100%). In hope that we might find a solvent more capable of improving our extraction efficient, we proceeded with an extended series of extractions by various solvents and by various methods. Among these methods was extraction with solvents (isopropanol, ethanol) recommended for our specific application by the company from which the resins had been purchased (Sorbent Technologies). None of these extractions, however, resulted in additional recovery.

At this point, we began to consider what might have caused a repression in extraction efficiency. One possibility was our use of DA-fortified DI-water (the FRS-UK group had only used DA-fortified seawater). Our next trial was, therefore, designed to repeat trial one except with the use of DA-fortified seawater.

Trial 3: DA-fortified filtered seawater (FSW); analysis by LC-MS

Trial three, and all subsequent trials (#4-6), were executed by the undergraduate associated with this Sea Grant project, Meiling Roddam. The purpose of trial three was therefore considered two-fold: (1) to introduce M. Roddam to the resins and familiarize her with their behavior, and (2) to generally assess whether the use of DA-fortified seawater affected extraction efficiency.

Because we were now soaking in DA-fortified seawater, it became necessary to SAX-clean all samples prior to their analysis by LC-MS. In a control sample with DA-fortified DI-water, we observed that DA loss due to SAX treatment could be as high as 30%. While SAX treatment of the adsorption samples to be analyzed by LC-MS are necessary in laboratory trials with DA-fortified seawater, it becomes unnecessary with extraction samples so long as the resins are rinsed with DI-

water prior to their extraction. Of course, this also requires that the resin be resistant to any inadvertent extraction of DA into a DI-water rinse (as demonstrated by the HP20 resin).

In this trial, the HP20 resin demonstrated poor adsorption properties, and was not extracted. Extraction of the SP700 resin was more successful in this trial than in the last, suggesting that the use of DA-fortified seawater was somewhat significant in terms of resin chemistry and behavior. We still, however, had not approached the extraction efficiency achieved with the SP700 resin by the FRS-UK group.

In a conversation with the FRS-UK group, it became clear that in their trials the SP700 resin had not been activated prior to use. We had thus far trialed only activated SP700 resins; this became a suspected factor in our repressed recovery of DA from the SP700 resin, and all SP700 resin in the subsequent trials (4-6) was interrogated in its unactivated state.

Trial 4: DA-fortified filtered seawater (FSW); analysis by LC-MS; 25g SP700 batch trial

In our correspondence with the FRS-UK group, we proposed a resin-swap and inter-lab comparison of extraction efficiencies; Liz Turrell and her colleagues in the FRS-UK group welcomed this proposal. A 25g (dry weight) batch of free unactivated SP700 resin was incubated in DA-fortified seawater until 100% adsorption had been achieved. The resin was then rinsed with DI-water (rinse was analyzed and showed no DA) and the resin batch was split into 5g (wet weight) portions. Two of these portions were extracted by M. Roddam per the protocol supplied by the FRS-UK group (provided below). One portion was sent to the FRS-UK lab for extraction per the same protocol. One portion was extracted by M. Roddam in a manner suggested by a colleague in our chemistry department (1 hour sonication in 50% MeOH followed by 23 hour soak, repeated in succession three times).

The FRS-UK protocol, in brief, is as follows:

- Place resin in a plastic column with ~20•m frit. Place on a vacuum manifold with the stopcock closed.
- Add 10mL 50% MeOH.
- Vortex for 1 minute.
- Replace on vacuum manifold and collect extraction fluid.
- Flush with an additional 90mL 50% MeOH and collect in the same vessel with the 10 mL (this step has been modified by the FRS-UK group to only 10 additional mL).
- Remove 10mL of the extraction fluid and evaporate at 40°C using a TurboVap-LV nitrogen gas evaporator.
- Re-suspend evaporite (DA) with 1 mL of 50% MeOH and analyze by LC-MS.

The recoveries achieved with the various resin splits were:

1. Extraction by M. Roddam (FRS-UK protocol): 11%
2. Extraction by M. Roddam (FRS-UK protocol): 7%
3. Sonication-soak cycling: 7%
4. Extraction by FRS-UK (FRS-UK protocol): 8%

Trial 5: DA-fortified filtered seawater (FSW); analysis by LC-MS; multi-resin trial

The consistency of the results achieved in trial four, and the relatively poor recovery demonstrated by the SP700 resin, encouraged us to pursue alternative

resins. Two resins, SP207 and SP207SS had been recommended to us by Sorbent Technologies expressly for our purposes. Another resin, SM-2 ("Biobeads") was suggested by a colleague after it had demonstrated promising results with an analyte possessing chemical properties similar to those of DA.

The SP207 and SP207SS resins demonstrated very quick and complete adsorption of DA (100% adsorption in < 3 hours). The results from the Biobeads adsorption series were difficult to interpret, since there was a positive response (artificially increased DA signal and/or issues with the estimation of the initial DA concentration in those incubations).

The SP207 and SP207SS resins demonstrated extraction efficiencies comparable to the extraction efficiency demonstrated by the unactivated SP700 resin. This encouraging result, and the excellent adsorption capacities suggested by the preliminary adsorption results from this trial, encouraged us to execute the design of trial six: a higher-resolution, triplicate-case adsorption series with both of these newly identified resins.

It should be noted that the primary difference between the SP207 and SP207SS resins is the size of the resin beads. The SP207SS is a resin that is much finer that the others we had trialed up until this point, and has the consistency of dust. Because of its fine consistency, this resin proved particularly difficult to work with as a free resin. We expect to trial this resin within a bag design and in a sealed column in the near future.

Trial 6: DA-fortified filtered seawater (FSW); analysis by LC-MS; SP207 and SP207SS trial

A higher-resolution, in-triplicate adsorption series with the SP207 and SP207SS resins suggest that the larger SP207 has slightly poorer adsorption abilities and greater variability in its adsorption profile. The SP207SS resin demonstrated 100% adsorption in < 30 minutes.

Subsequent extraction of these resins was carried out first with 50% MeOH (resin in column, 10 mL 50% MeOH added, 1 minute vortex, collection of extract), followed by 1 M solutions of ammonium acetate and ammonium formate in 50% MeOH. We elected to try ammonium salt solutions for extraction to assess whether raising the pH improved extraction efficiency. With this extraction series, we were able to achieve 40-50% extraction efficiency.

Project outcomes

We are behind on our original timeline, because of the significant issues we needed to address regarding the extraction of the toxins from the various resins. However, we have an approximately 1-year time-series of field-deployed SPATT resins (HP-20 and SP-700), and are in the process of analyzing these samples. In conjunction with that time-series, we have the full weekly sampling conducted as part of Cal-PReEMPT, which includes nutrients, chlorophyll, cell counts, harmful algal species counts, sentinel mussel samples, and other data. All of the data are maintained in a database, and are provided to the California Department of Public Health and to the public upon request.

As part of this project we also instrumented the Santa Cruz Wharf to provide important ancillary information. This includes a publically available datastream from a weather station that is being co-maintained by our lab and the City of Santa Cruz. The weather station data have proven valuable for correlating cell and toxin values to the ambient conditions at the SC Wharf.

Impacts of project
We successfully tested the SPATT resins that have been used elsewhere in the world, for adsorption of several algal toxins. Perhaps the most important finding from our study is that there are serious issues with the methodology as previously published, but with some modification, these resins can be used to provide a passive sampling system. This is a first step towards addressing these problems is to develop new and better toxin and nutrient monitoring methodologies, to assess the occurrence and magnitude of these toxins, and to determine the relationship (if any) to nutrient loading.

This project supported a Sea Grant Trainee (Jenny Lane) and an undergraduate student (Meiling Roddam). Ms. Roddam gained valuable experience conducting her own research, and is currently working up the data as part of her Senior Thesis at UC Santa Cruz.

This project also helped to develop a better monitoring program at the SC Wharf, in collaboration with Cal-PReEMPT (NOAA MERHAB) and the City of Santa Cruz. We purchased and deployed an ISCO sampler, and purchased and deployed a WeatherHawk weather station, substantially improving this site. Based on these upgrades, the SC Wharf is being further upgraded with funds from CeNCOOS and UCSC. We expect that the SC Wharf will become an outstanding harmful algal bloom monitoring site and will be used to develop and test new methodologies. This would not have been possible, or at least would have been slowed down substantially, without Sea Grant funds.

Benefits, commercialization and application of project results
We have presented our preliminary results to California Department of Public Health, to CCLEAN, and to the California Department of Fish and Game. These groups are interested in further pursuing the SPATT methodology. At this time, they are still waiting for us to complete our project and provide guidelines for the use of these resins.

CDPH: Gregg Langlois, CDPH, 850 Marina Bay Parkway, G165, Richmond, CA 94804. Gregg.Langlois@cdph.gov.

CCLEAN: Dane Hardin, Director of CCLEAN, PO BOx 8346, Santa Cruz, CA 95061. hardin@amarine.com.

CDFG: Melissa Miller, MWVCRC, Department of Fish and Game, 1451 Shaffer Road, Santa Cruz, CA 95060. mmiller@ospr.dfg.ca.gov

Economic benefits generated by discovery
We believe there could be substantial economic benefits if the SPATT resin proves to be effective. It is less expensive than maintaining sentinel mussels, and is not impacted by changes in salinity. There are broader economic advantages as well, related to the reduction of economic loss due to shellfish toxins, that are difficult to quantify.

Issue-based forecast capabilities
At this time, we do not have forecast capabilities based on the SPATT resin. Our ultimate goal is to link toxin presence to environmental conditions, which would provide the ability to predict or forecast toxin events. An example of this, using the same data collected weekly from the Santa Cruz Wharf, was accepted for publication by Jenny Lane:

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 (accepted).

Tools, technologies and information services developed
The main purpose of this proposal was to evaluate the SPATT resin, which could be considered a passive environmental sensor.

Publications
Conference papers, proceedings, symposia
Title: A summary of HAB Monitoring, Alert, and Event Response in California
Authors: Raphael Kudela
Date: 10 February 2009
Conference Title: West Coast Regional Harmful Algal Bloom Summit
Location: Portland, OR

Please list any workshops/presentations given
The results from our project were presented as part of the West Coast Regional Harmful Algal Bloom Summit held 10-12 February, 2009 in Portland, OR. There were about 100 attendees. The audience was researchers, managers, and educators working on HABs on the US west coast. As part of the summit, we presented preliminary results from the SPATT resin trials.

Students
Jenny Q. Lane
University of California Santa Cruz
Department of Ocean Sciences
Degree program enrolled in: Ph.D.
Theses/dissertation title: A toxigenic Pseudo-nitzschia bloom model, leading to reconsideration of eutrophication processes within the contexts of seasonality and temporal relativity
Supported by Sea Grant funds? [x] yes [] no
Start date: 3/1/2008
End date: 2/28/2009

Meiling Roddam
University of California Santa Cruz
Department of Ocean Sciences
Degree program enrolled in: Marine Biology BS
Theses/dissertation title
Supported by Sea Grant funds? [] yes [x] no
Start date: 03/01/2008
End date: 02/28/2009

Cooperating organizations
Federal
NOAA MERHAB Program. They provided logistical support through an ongoing project (availability of related environmental data)

Regional
Central and Northern California Ocean Observing System; provided funds for maintenance and upgrade of the Santa Cruz Wharf sampling site

Local and state
City of Santa Cruz; provided access to, and logistical support, for the Santa Cruz Wharf sampling site

International implications
We collaborated with Dr. Elizabeth Turrell, Biotoxins and Applied Microbiology Group Leader, Fisheries Research Service, UK. Dr. Turrell has been evaluating the same methods that we used as part of our project, and is also deploying the passive resins internationally. We shared methodologies and samples between the two labs.

Keywords
harmful algae, domoic acid, yessotoxin, saxitoxin, microcystin, passive toxin absorption, amnesiac shellfish poisoning, coastal monitoring, Pseudo-nitzschia, Microcystis