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Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California

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Abstract

Recent publications have identified the analysis of phycotoxins in sentinel shellfish as a problematic tool for environmental monitoring purposes. Domoic acid (DA), a neurotoxin produced by some species of the diatom *Pseudo-nitzschia*, can remain undetected in sentinel shellfish stocks during toxic blooms and subsequent marine bird and mammal mass mortality events. Solid Phase Adsorption Toxin Tracking (SPATT) has previously been described for monitoring of lipophilic toxins, whereas resin-based sampling methods are routinely employed for many other environmental contaminants. Here, we evaluate the applicability of SPATT for monitoring the hydrophilic phycotoxin DA and demonstrate that the same field sampling methods can be used for the detection of saxitoxins. We present laboratory-based adsorption profiles characterizing the performance of SPATT with four resin types: (1) HP20, (2) SP700, (3) SP207, and (4) SP207SS. We present results from 17 mo of approximately weekly SPATT deployments in Monterey Bay, California (USA); this period included two significant toxigenic *Pseudo-nitzschia* bloom events as well as low-level saxitoxin events. SPATT signaled the presence of DA 3 and 7 weeks before the recognition of bloom conditions by traditional monitoring techniques (7 and 8 weeks before shellfish toxicity). Under ambient (non-bloom) conditions, all resins detected DA when its presence was not apparent from traditional monitoring, highlighting the ubiquity of low level or transient toxin events in the environment. This study is the first to evaluate SPATT deployments in U.S. waters, and the first to demonstrate the applicability of SPATT toward detection of hydrophilic phycotoxins in the field.

The contamination of shellfish with phycotoxins is a public health risk encountered worldwide. In many countries, shellfish product safety is assured through biotoxin monitoring programs. In recent literature, the analysis of shellfish for biotoxins has been described as difficult, expensive, problematic, time-consuming, technically demanding, and "not ideal as a tool for monitoring the progress of toxigenic blooms" (Fux et al. 2009; Mackenzie et al. 1993; Mackenzie et al. 2004; Mackenzie 2010; Rundberget et al. 2007). Although direct monitoring of shellfish for biotoxins has obvious benefits and unique significance, disadvantages include (1) an analytical process regarded as time-consuming, expensive, technically demanding, and labor-intensive, (2) analytical interferences resulting from biological matrix effects, (3) heterogeneity inherent to the employment of a biological matrix, (4) toxin bio-transformation and toxin depuration, both of which confound toxin detection and quantification, and (5) inability to control stock supply; years of low shellfish recruitment can translate to a lack of shellfish stocks from, or available for transfer to, sentinel observation sites. Phytoplankton monitoring, often instated in conjunction with shellfish monitoring, offers valuable insight into the ecology and development of toxic blooms but is limited in its ability to signify biotoxicity in the target organisms because (1) phytoplankton samples can only describe a 'snapshot' of the phytoplankton assem-

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blage at a single point in space and time, (2) observation of a phytoplankton assemblage can provide only circumstantial evidence for the possibility of toxin accumulation, and (3) positive identification to the species level is difficult for some toxigenic algae such as the genus *Pseudo-nitzschia*; a monitoring agency risks either increased incidence of false alarm or must further invest in, or develop, specialized tools required for species-level identification of these groups (Mackenzie et al. 2004; Mackenzie 2010; Miller and Scholin 1996, 1998, 2000; Rundberget et al. 2009).

The use of a passive sampling method for determination of dissolved biotoxin levels in seawater, Solid Phase Adsorption Toxin Tracking (SPATT), was first proposed in 2004 as a means by which the disadvantages associated with shellfish could be circumvented (Mackenzie et al. 2004). SPATT extended the varied list of analytes and mediums toward which passive samplers had been applied (see reviews in Górecki and Namieśnik 2002; Kot-Wasik et al. 2007; Seethapathy et al. 2008; Vrana et al. 2005). As first described, SPATT is the field-deployment of adsorbent resin sealed within a polyester mesh bag. SPATT bags of this design were tested by Mackenzie et al. (2004) for a suite of lipophilic toxins, including the pectenotoxins (PTX 2, PTX 2 SA, PTX 11, PTX 11 SA), the okadaic acid complex toxins (OA, OA-ester), dinophysis toxin-1 (DTX 1), and yessotoxin (YTX). Studies subsequent to Mackenzie et al. (2004) focused on the development of alternative sampler designs with DIAION® HP20, the resin identified as the most efficient for lipophilic toxin tracking; relatively fewer studies discussed the potential of alternative resins (Table 1). In all published studies to date, SPATT has targeted lipophilic toxins exclusively for quantification. This study is the first to evaluate semi-quantitative use of SPATT for field monitoring of the hydrophilic neurotoxin domoic acid (DA; Fig. 1) as well as saxitoxin (STX) and related hydrophilic paralytic shellfish toxins (collectively referred to as PST), produced by species of the diatom genus *Pseudo-nitzschia* and the dinoflagellate genus *Alexandrium*, respectively.

DA toxicosis manifests as Amnesic Shellfish Poisoning (ASP) in humans, and is also referred to as Domoic Acid Poisoning (DAP; particularly for wildlife intoxication) to distinguish between shellfish and other vectors (Scholin et al. 2000). The first reported outbreak of ASP occurred in 1987 when 3 people died and over 100 were hospitalized following the consumption of contaminated shellfish from Prince Edward Island, Canada (Bates and Trainer 2006). The California Department of Public Health (CDPH) Marine Biotoxin Monitoring Program included DA in their routine monitoring program in 1991, following identification of DA along the California coastline (Fritz et al. 1992). Since 1987, no human deaths have been attributed to ASP in California; DA poisoning has, however, been identified as the causal factor in mass mortality events involving marine mammal and bird populations (Schnetzer et al. 2007; Scholin et al. 2000). While the incidence of DA and its bio-accumulation in the food web is a public health concern first and foremost, the sudden or unan-

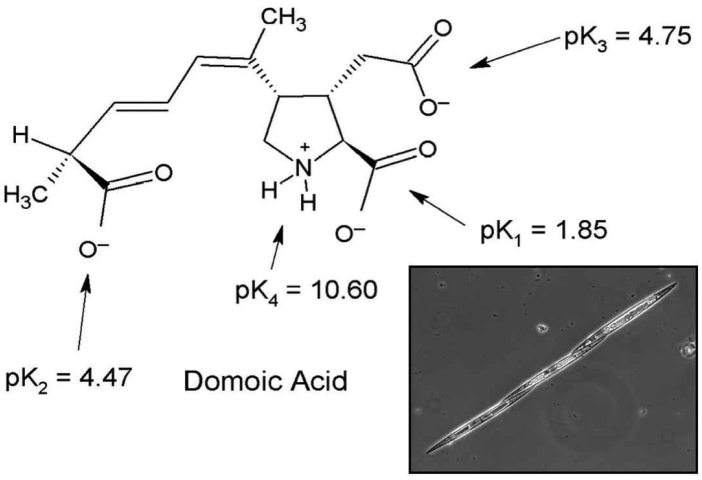


Fig. 1. The molecular structure of domoic acid, as protonated in seawater. Inset: a 3-cell chain of the diatom *Pseudo-nitzschia*.

Table 1. Published studies on the employment of Solid Phase Adsorption Toxin Tracking (SPATT). Phycotoxin abbreviations are as follows: dinophysistoxin (DTX), okadaic acid (OA), pectenotoxin (PTX), yessotoxin, (YTX), gymnodimine (GD), azaspiracid (AZA), spirolide (SPX), domoic acid (DA), saxitoxin, and related paralytic shellfish toxins (PST). Bold text indicates the resin identified as optimal for the toxins addressed in the study.

Study	Toxin group analyte	Resins	Region	Mode of deployment
MacKenzie et al. (2004)	DTX, OA, PTX, YTX	**HP20**, SP207, HP2MG	New Zealand	Sewn bags
Takahashi et al. (2007)	GD, OA, PTX	HP20	Australia	Sewn bags
Rundberget et al. (2007)	DTX, OA, PTX	HP20	Norway, Spain	Packed columns
Turrell et al. (2007)	OA, PTX, YTX, AZA	HP20, **SP700**	Ireland	Zip-tied mesh bags
Turrell et al. (pers. comm.)	DA	HP20, **SP700**	Ireland	Zip-tied mesh bags
Pizarro et al. (2008a, 2008b)	DTX, OA, PTX	HP20	Spain	PVC frame
Fux et al. (2008)	DTX, OA	**HP20**, SP850, SP825L, XAD4, L-493	Ireland	Sewn bags, Embroidery disc
Fux et al. (2009)	DTX, OA, PTX, YTX, AZA, SPX	HP20	Ireland	Embroidery disc
Rundberget et al. (2009)	OA, PTX, YTX, AZA, SPX	HP20	Norway	Embroidery disc
This study	DA, PST	HP20, SP700, SP207, SP207SS	USA (California)	Heat-sealed bags

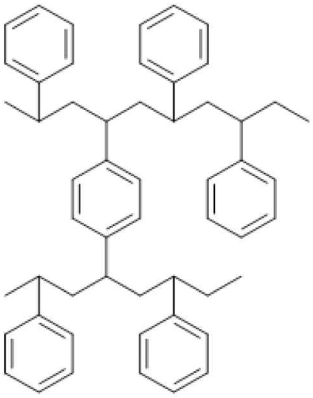
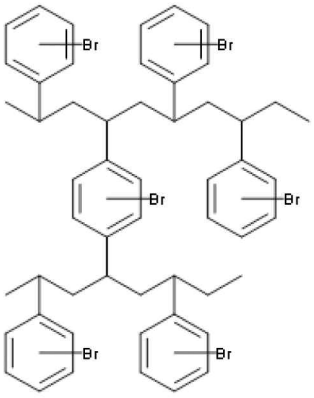
ticipated onset of a DA poisoning event within wildlife populations can prove detrimental through secondary impacts afflicting areas unrelated to human health (e.g., ecologic, economic, aesthetic).

Here, as in preceding studies, SPATT is prescribed and evaluated as a monitoring technology for time-integrative detection of dissolved toxin (Mackenzie et al. 2004; Mackenzie 2010; Pizarro et al. 2008a, 2008b; Rundberget et al. 2009; Takahashi et al. 2007; Turrell et al. 2007) and is assessed for its potential to provide "reliable, sensitive, time-integrated sampling to monitor the occurrence of toxic algal bloom events" (Mackenzie 2010). We evaluate the applicability of SPATT for the detection of DA in seawater, in both controlled-condition laboratory trials and coastal site field deployments. We compare SPATT to both particulate DA and sentinel shellfish data; whereas SPATT only detects toxins in the dissolved phase, previous publications have discussed the prevalence of dissolved versus particulate or bio-accumulated toxins during harmful algal bloom events (e.g., Mackenzie et al. 2004), and we do not further address this point herein. We specify four candidate SPATT resins and describe their DA-adsorption efficiencies under controlled conditions. We present an adapted extraction protocol that provides rapid recovery of DA from SPATT bags deployed in the field, and describe extraction efficiencies obtained under controlled conditions. We present a new method of SPATT passive sampler construction that minimizes labor and skill requirements, while allowing for the manufacture of a pliable sampling device. In the field trial component of our study, SPATT samplers of this new design were deployed approximately weekly over a 17-mo period in Monterey Bay, California (USA) in conjunction with weekly monitoring of DA in sentinel shellfish stocks, particulate DA (pDA), chlorophyll-*a*, phytoplankton abundance, and local oceanographic conditions. We present results from these field deployments, which encompassed two significant bloom events of toxigenic *Pseudo-nitzschia* in spring and fall 2009. As a secondary effort, a subset of extracts from the HP20 resin SPATT deployments were selectively analyzed for identification of STX and closely related compounds, demonstrating the ability to successfully detect multiple analytes from a single field-deployed SPATT device. These data are presented in conjunction with measurements of Paralytic Shellfish Toxins (PSTs) in sentinel shellfish as reported by the California Department of Public Health (CDPH) and cell concentrations of *Alexandrium catenella,* the presumed causative agent of PST in California coastal waters.

Materials and procedures

All macroporous resins (DIAION® HP20 and SEPABEADS® SP700, SP207, and SP207SS) were purchased from Sorbent Technologies, USA. The properties of these resins are summarized in Table 2. Water (Fisher W74), acetonitrile (Fisher A955), and methanol (MeOH; Fisher A456) used in liquid chromatography-mass spectrometry (LC-MS) analysis were purchased as Optima LC-MS grade from Fisher Scientific, USA.

Table 2. The physical characteristics of the resins evaluated for SPATT in the present study.

Resin	HP20	SP700	SP207	SP207SS
Structure	Polystyrene-divinylbenzene (PS-DVB)		Modified PS-DVB	
				
Water content (%)	55-65	60-70	43-53	
Particle size (μm)	>250		>250	75-150
Pore volume (mL g^{-1})	1.3	2.3	1.3	
Surface area (m^2 g^{-1})	600	1200	630	
Pore diameter (Å)	520	180	210	
Specific gravity (g mL^{-1})	1.01	1.01	1.18	

Ammonium acetate (≥ 97%; MP Biomedicals 193848), formic acid (≥ 99%; Acros Organics AC27048-0010), isopropanol (Fisher A4644), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA; Acros Organics AC32720-1000), and MeOH (HPLC grade; Fisher A452) used in SPATT extractions were purchased from Fisher Scientific, USA.

DA analysis was conducted using an Agilent 6130 LC-MS system with an Agilent Zorbax Rapid Resolution column using an 8-point dilution series of CRM DA-f domoic acid standards for calibration. The LC-MS was operated with a gradient elution of acidified water (0.1% formic acid) and acidified acetonitrile (0.1% formic acid). The same certified DA standard, obtained from the National Research Council (Canada) was used in LC-MS calibrations and incubation medium fortifications (NRC CRM DA-f). Standards used the same extraction matrix as the samples (50% MeOH or 50% MeOH with 1 M ammonium acetate). DA was identified by the presence of a 312 amu peak in positive Scanning Ion Mode (SIM) with concentration determined by signal integration of the peak area and back-calculations based on the standard curve. Our limit of detection was equal to or better than 34 pg DA on-column. A modified solid phase extraction (SPE) clean-up procedure (Wang et al. 2007) was applied to fortified seawater samples collected for adsorption profiling. In laboratory trials, the percent DA adsorbed and percent DA recovered were determined by mass balance; since no loss of DA was observed in the experimental control incubations, the loss of DA from the treatment incubations was attributed to the adsorption of DA by the experimental treatment (SPATT).

For a subset of samples, SPATT extracts were analyzed for the presence of STX and related compounds using Abraxis Saxitoxin ELISA kits (PN 52255B) using a modification of the manufacturer's directions. Briefly, SPATT extracts (in 50% MeOH) were either diluted in buffer solution or plated directly. Although the kits are not designed for analysis of MeOH extracts, verification using Abraxis STX standards with 50% MeOH exhibited no matrix issues (data not shown). Abraxis reports 100% cross-reactivity for STX, with varying sensitivity (<0.2% to 29%) for neosaxitoxin, gonyautoxins (GTX), and derivatives.

To determine resin saturation values for DA, known quantities of resin (HP20 and unactivated SP700) were exposed to excess DA in Milli-Q for 45 d, and total adsorption was determined by mass balance to be 7300 ng g^{-1} and 17000 ng g^{-1}, respectively. A second (non-saturating) trial was performed with known quantities of resin (non-activated SP700, activated SP207, and activated SP207SS; $n = 3$ for each treatment) exposed for 8 d to DA in artificial seawater to better simulate field deployments, providing values of 8083 (CV = 20%), 8541 (CV = 13%), and 19,644 (CV = 7%) ng g^{-1} resin, respectively. PST saturation values were not determined as part of this study. Using a conservative value of 7300 ng g^{-1} for HP20 and assigning 3 g SPATT deployments, we would expect saturation to occur at ~21,900 ng DA; the highest recorded value from our time series was 182 ng g^{-1} (546 ng DA). While 3 g deployments were determined sufficient for our field purposes, we note that during extreme bloom events, it is possible the various resins can saturate during a 7-d deployment, and users may wish to adjust the amount of resin or the duration of deployment if this is a concern.

Sampler design and construction—A new method of SPATT bag construction was developed and applied. Unlike previously described construction methods (Table 1), the new method afforded quick manufacture of sealed, pliable SPATT bags without any requirement for specialized skills or equipment not generally found among oceanographic cruise supplies. As such, these bags could be quickly manufactured for deployment across a variety of configurations (e.g., narrow-neck flasks in laboratory trials, clipped into an embroidery hoop for field-deployment). SPATT bags were constructed from 100 µm Nitex bolting cloth (Wildlife Supply Company, Product No. 24-C34, $45/yd). The bolting cloth was sealed on three sides using a plastic bag sealer (Clamco® Impulse Sealer, Model 210-12E) to form an open bag of 55 mm width. The bag was filled with 3 g (dry weight) resin and the fourth side sealed with the bag sealer to form a finished SPATT bag of 55 × 55 mm dimension. For activation, SPATT bags were soaked in 100% MeOH for 48 h, then rinsed thoroughly in de-ionized water (Milli-Q) and transferred into a fresh volume of Milli-Q for removal of MeOH residues by sonication using a probe sonicator (Fisher Scientific® Sonic Dismembrator, Model 100). The bags were stored in Milli-Q at 4-6°C prior to use.

Adapted extraction protocol ('UCSC field protocol')—An unpublished extraction protocol for use with SP700 field-deployed SPATT resin was provided by the Fisheries Research Services [FRS], Marine Laboratory (Aberdeen, UK), and adapted for its application toward the extraction of field bags recovered by the University of California Santa Cruz (UCSC). Per the adapted protocol (hereafter referred to as the 'UCSC field protocol'), the SPATT bag is rinsed with 3× ~200 mL Milli-Q and inserted whole into a 1.5 × 12 cm polypropylene chromatography column with a porous 30 µm polyethylene bed (Bio-Rad, Cat No. 732-1010), and 10 mL of 50% MeOH (v/v) is added. The column is vortexed for 1 min, placed on a vacuum manifold, and the extract is eluted into a glass scintillation vial. The extraction sequence is repeated with 10 mL ammonium acetate in 50% MeOH (1 M). The extraction sequence is repeated a final time with 10 mL ammonium acetate in 50% MeOH (1 M). The column and bag are rinsed with 10 mL ammonium acetate in 50% MeOH (1 M), and this rinse is collected as part of the final extraction. Each fraction is collected separately and analyzed; only the first extract (50% MeOH) was used for our determination of STX and related PSTs due to concerns about interference from the ammonium acetate in subsequent extracts. We did not concentrate the extracts before analysis by LC-MS due to concerns about DA stability (e.g., Wang et al. 2007), but inclusion of an evaporative step would increase the minimum detection limit proportionally to the concentration.

Evaluation of adsorption and extraction efficiency—The adsorption profile for SPATT bags of each resin type were determined by laboratory trial. SPATT resin bags were incubated, in triplicate, in aliquots of DA-fortified filtered seawater (or Milli-Q, where specified) in 125 mL glass flasks with rubber stoppers. The incubations were maintained at a controlled temperature (15°C) with constant agitation (70 rpm) with 12:12 illumination using "cool white" lamps at approximately 125 µmol photons m^{-2} s^{-1}. The sample water was regularly assayed for DA to monitor adsorption by the resin. The SPATT bags used for the determination of adsorption efficiencies were subsequently extracted according to the UCSC field protocol.

While DA could be extracted readily from HP20 (> 99% extraction efficiency when accounting for DA loss to Milli-Q rinses), extraction inefficiencies demonstrated for the other resins (2% to 11% extraction) prompted a secondary investigation into our extraction capacity with application of more extensive extraction techniques. A batch (9.200 g) of non-activated SP700 was soaked in DA-fortified Milli-Q (60 mg mL^{-1} CRM DA-f) until DA in the incubation medium was below detection by LC-MS. The resin was then split into 4 batches (1.145, 1.192, 1.006, and 5.572 g, respectively). Resin batches 1, 2, and 3 were extracted with 50% MeOH, 50% MeOH with EDTA (10 mM), and ammonium acetate in 50% MeOH (1M), respectively, as follows: (1) 7 d soak, then elution/analysis, and (2) addition of fresh solvent, with elution/analysis on day 18. The solvents for resin batches 1 and 2 were reserved and used to extend the soak/extraction period; additional elution/analyses were performed at 29 d and 41 d. Resin batch 4 was packed into a Restek stainless steel column (250 × 4.6 mm), installed as an HPLC column, and run at 2 mL min^{-1} with the following series of solvents: acidified water (0.1% formic acid; 40 mL), 50% MeOH (22 mL), 90% MeOH (18 mL), isopropanol (30 mL), 50% MeOH (10 mL), 50% MeOH with ammonium acetate (1M; 20 mL), and 50% MeOH (20 mL). The fortified Milli-Q was kept under the same storage conditions (room temperature, low light) and analyzed at each time point to check for degradation of the DA (none was observed).

The consistency of adsorption and extraction performance in field bags was investigated through the field-deployment of replicate SPATT bags and their extraction per the UCSC field protocol. SPATT bags of HP20, non-activated SP700 and SP207 were field-deployed [*see* "Field deployment at Santa Cruz Municipal Wharf (SCMW)," next section] in triplicate for 10 d (18–28 Nov 2009). The bags were processed as field bags (rinsed in Milli-Q, stored at –80°C) and extracted per the UCSC field protocol on 02 Dec 2009.

Field deployment at Santa Cruz Municipal Wharf (SCMW) — The resins HP20 and SP700 were evaluated in weekly field deployments at SCMW (36° 57.48′N, 122° 1.02′W) from 15 Jul 2008–01 Dec 2009; for 7 of these rotations, the deployment period exceeded 9 d. Deployment of SP700 as a nonactivated resin began on 29 Apr 2009, and the resin SP207 (non-activated) was added to the field rotations on 20 May 2009. For deployment, SPATT bags were clamped into plastic embroidery hoops (Susan Bates® HOOP-La, 7.6 cm dia), and secured to a weighted rope with a plastic zip-tie at approximately 2.5 m depth. For SPATT bag retrieval and transport, the bag was unclamped from the embroidery hoop, immediately rinsed in ~200 mL Milli-Q, and transported to the lab in a glass container on ice. Upon arrival, the bag was rinsed twice more in Milli-Q (2 × 200 mL), then placed in a 20 mL glass scintillation vial and stored at –80°C. All archived bags were thawed entirely prior to extraction. Toxin values are reported normalized to 1 g resin and length of deployment (e.g., ng DA per gram resin per day) and were not corrected for estimated extraction efficiency.

SPATT bags were deployed at the same depth as bags of sentinel mussels (~2.5 m) maintained and sampled as part of the CDPH Marine Biotoxin Monitoring Program. Net-tow [5 × 10 ft vertical effort; 20 µm mesh with net dimensions 25 × 100 cm (leading diameter × length) and a 5 × 20 cm cod-end (internal diameter × length)] and whole-water samples were collected near the deployment site for qualitative evaluation of the phytoplankton assemblage (presence/absence and estimate of relative abundance to the genus level) and for the quantification of toxigenic *Pseudo-nitzschia* species (*P. australis* and *P. multiseries*) and *Alexandrium catenella*. Relative abundances of phytoplankton were determined with a Leica MZ125 stereomicroscope within 1 h of sample collection. Whole water was collected by integration of water samples taken from 3 discrete depths (0, 1.5, and 3 m) with a FieldMaster 1.75 L basic water bottle. *Pseudo-nitzschia* and *Alexandrium* species identification and enumeration used species-specific large subunit rRNA-targeted probes following standard protocols (Miller and Scholin 1998). Samples were enumerated with a Zeiss Standard 18 compound microscope equipped with a fluorescence Illuminator 100 (Zeiss). Duplicate filters were prepared for each species, and the entire surface area of each filter was considered in counting.

Assessment

Adsorption profiles—The DA adsorption profiles of the candidate resins in SPATT bags are characterized in Table 3 and are presented in Fig. 2. The profiles are two-phase exponential decay curves fit to time-point observations of DA concentration in the incubation medium (normalized to initial DA concentration) except for the adsorption profile of HP20 in seawater, which is best fit by linear regression (Fig. 2B). The estimated adsorption curves were poorest for HP20 in both Milli-Q and in seawater (R^2 = 0.82 and 0.55, respectively). The adsorption curves of the SP700, SP207, and SP207SS resins fit the data well (R^2 > 0.90; Table 3). Adsorption profiles are presented for both activated and non-activated SP700 SPATT bags (Fig. 2B), since both were employed as part of our field deployment program. Through 192 h (8 d), there is no difference between the adsorption behavior of activated and non-activated SP700 (2-way ANOVA; P = 0.11); the difference becomes

Table 3. Adsorption characteristics for the SPATT resins evaluated in the present study. In all cases described here, domoic acid (DA) adsorption was from DA-fortified 0.2 µm-filtered seawater. Comparable results (not tabulated) were obtained for DA-fortified Milli-Q water (e.g., Fig. 2A).

	HP20	SP700 (non-activated)	SP700 (activated)	SP207	SP207SS
Adsorptive character	Slow/weak	Moderate/moderate	Moderate/strong	Fast/strong	Fast/strong
Adsorption in 7 d (%)	19	64	70	97	93
Time to full adsorption (d)	36	22	17	13	10
Profile fit (R^2)	0.55	0.93	0.99	0.94	0.91

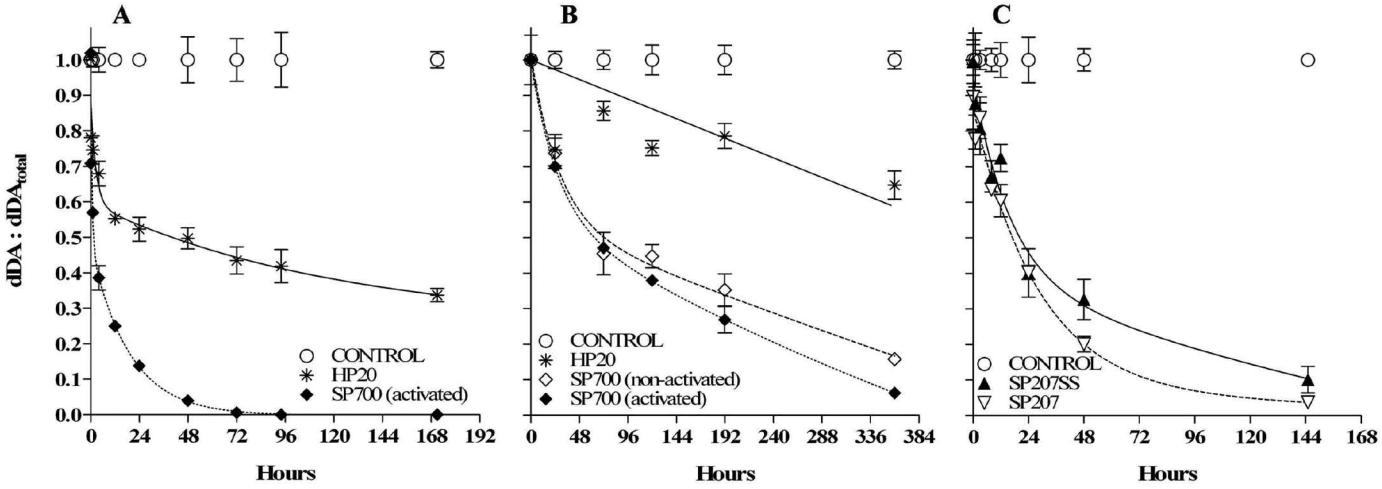


Fig. 2. Adsorption profiles for SPATT bags of the candidate resins assessed for application toward domoic acid (DA): (A) HP20 and SP700 in DA-fortified Milli-Q; (B) HP20 and SP700 in DA-fortified seawater; (C) SP207 and SP207SS in DA-fortified seawater. All adsorption profiles are presented as two-phase exponential decay curves except for the linear fit presented for HP20 in seawater (B). All exponential decay curves were significant ($R^2 > 0.9$, $P < 0.05$; see Table 3).

significant when the adsorption curve is extended to 360 hours (15 days; $P = 0.02$).

Extraction efficiencies—Instances of incomplete adsorption from the medium (e.g., HP20, Fig. 2) confounded the evaluation of extraction efficiencies across resin types. Regardless, HP20 could be distinguished as a relatively 'leaky' resin; in preliminary trials, our handling of HP20 bags per the original (FRS) extraction method indicated the unintended 'extraction' (loss) of DA from the bags into pre-extraction Milli-Q rinses. While the leaky character of HP20 precludes a concise description of its extraction efficiency, losses of DA from HP20 field deployments into the pre-extraction Milli-Q rinses prescribed by the UCSC field protocol could be: (1) accounted for through the re-designation of the Milli-Q rinses as 'extractions,' which are then reserved, cleaned on an SPE column, and analyzed, or (2) encountered as a consistent loss term across HP20 field deployments, which are handled and extracted according to the same field protocol. Compared with the HP20 resin, the three alternative resins (SP700, SP207, SP207SS) were relatively aggressive in their retention of DA, and our DA recovery efficiencies from the experimental (non-HP20) SPATT bags were comparatively low (2% to 11%). The application of more exhaustive extraction techniques toward a DA-loaded batch of free non-activated SP700 resin demonstrated the capacity to recover DA from these resins exhibiting strong binding characteristics, but at higher analytical and material cost and with substantially longer time intervals between deployment and final analysis. Soak-extraction of the SP700 resin in 50% MeOH and 50% MeOH with EDTA (10 mM) yielded 47% and 52% recovery on day 7; cumulative recovery increased with prolonged soaking (61% to 62%, 68% to 69%, and 71% to 72% on day 18, 29, and 41, respectively). Soak-extraction in 50% MeOH with ammonium acetate (1M) demonstrated lower cumulative recovery (5% to 10% on day 7, 34% on day 18). Packing DA-loaded (non-activated) SP700 into an HPLC column for pressurized extraction yielded 15.4%, 78.6%, and 82% cumulative recovery with the first 3 solvents used for column elution (acidified water, 50% MeOH, 90% MeOH) with no additional recovery of DA by solvent elutions thereafter, nor with removal of the resin from the column and 50% MeOH soak for an additional 10 d. Since we estimate recovery based on mass balance, we cannot determine whether the remaining DA (18% to 29%) was lost from the SP700 resin during processing, was still adsorbed, or was degraded. In consideration of loss to degradation, we note the >99% recovery from HP20, a similar resin (Table 2); this recov-

ery efficiency suggests that DA is not inherently unstable when exposed to resin. Although we did not extensively test storage effects as part of this study, our ad hoc results indicate that the resins and extracts (50% MeOH) are stable for at least several months with no loss or transformation of DA. Other extraction solvents and protocols were evaluated, including longer chain alcohols (ethanol, isopropanol) of varying strength, pH-adjusted methanolic solutions (both acidic and basic), sonication, heating, and warm (40°C) bath sonication (data not shown), with no improvement in extraction efficiency.

While adsorption profiles and extraction efficiencies can be estimated and described in the experimental setting, these data cannot be directly extrapolated to the field setting and presumed to hold constant in an uncontrolled environment. We therefore directly evaluated the variability inherent to our SPATT bag field deployment/extraction method through a deployment rotation of triplicate bags in the field; the coefficients of variation (CV) were 14.9% for HP20, 36.9% for SP700 and 15.8% for SP207. The relatively high CV for SP700 was caused by an outlier within the SP700 bag extractions. For comparison, an analogous assessment of variability among individual sentinel mussels (n = 12) determined CVs of 40.6% and 45.6% for analysis by LC-MS and Biosense ELISA, respectively, following standard extraction protocols and analysis in our laboratory.

Field deployment—The results of SPATT bag field deployment at SCMW are presented with *Pseudo-nitzschia* relative abundance (indexed by visual inspection of the sample and evaluation of the phytoplankton assemblage at the genus level; Fig. 3A), DA in shellfish (Fig. 3B), cell counts specific to toxigenic species of *Pseudo-nitzschia* (Fig. 3C), and particulate DA (Fig. 3D). While the toxigenic species *P. australis* and *P. multiseries* have been associated with toxic bloom events observed in Monterey Bay, their recognition as locally toxigenic species does not eliminate the potential for DA production by species other than *P. australis* and *P. multiseries*; for this reason, we present the SPATT results with both discrete cell counts of these toxigenic species and the index of relative abundance at the genus level. While we consider SPATT to be semi-quantitative because we can't directly relate resin toxin loads to quantitative toxin concentrations in the environment, field deployments used identical procedures, and are therefore internally comparable (i.e., low/high values for a given resin type are assumed to indicate low/high values in the environment). Of the three resins used in field testing, HP20 was the only resin deployed consistently (always activated) throughout the 17 months deployment period. The HP20 deployments successfully signaled the presence of DA periodically throughout the year. A significant episode of SPATT-DA signaling by HP20 began in late February (deployment period 25 Feb – 03 Mar 2009) and had surpassed all previous signal magnitudes by mid-March. Toxigenic *Pseudo-nitzschia* and pDA were detected on 21 Apr 2009, 6 weeks after the first week-rotation of SPATT-DA signaling. Toxigenic *Pseudo-nitzschia* were identified at 'bloom-level' concentrations (>10,000 cells L^{-1}) on 05 May 2009, and shellfish toxicity was detected on 11 May 2009, 7 and 8 weeks, respectively, after the first rotation of elevated SPATT-DA signaling. Non-activated deployments of SP700 began on 29 Apr 2009 (near to the observed bloom-peak in pDA concentration), and SP207 deployments began on 20 May 2009 (near to the end of the bloom period). The DA-signals from SP700 were initially high, and then SPATT-DA signaling from all three resins declined in conjunction with, or in slight advance of, indicators afforded through traditional monitoring (i.e., cell counts and particulate toxin monitoring).

All three SPATT resins dropped to low and/or zero detection for three weekly rotations following the spring 2009 bloom event. Summer 2009 was a period of intermittent SPATT-DA signaling and disjointed signaling by traditional monitoring techniques (e.g., pDA detection without cell detection and vice-versa; single-week detection incidents). The SP700 and SP207 SPATT resins signaled DA across two weekly rotations in late June/early July 2009 (HP20 signaled across only the latter of the two rotations). This signaling event may be recognized in the pDA record as a single detection incident (23 ng L^{-1} on 23 Jun 2009). Particulate DA (and toxigenic *Pseudo-nitzschia* cell counts) remained below detection for the remainder of the summer, throughout a second summer period of sustained SPATT-DA signaling (15 Jul–18 Aug 2009). SPATT DA-signals then fell to zero over a two-week period of low *Pseudo-nitzschia* relative abundance and low (<1000 cells L^{-1}) toxigenic *Pseudo-nitzchia* species abundance; both traditional measures of toxin incidence (pDA and DA in shellfish), indicated this as a period of non-toxicity (no detection of DA in the particulate fraction or in shellfish). SPATT-DA began a consistent signal-response to the impending fall bloom on 02 Sept 2009, 3 weeks prior to the detection of 'bloom' conditions (>10,000 cells L^{-1}), and 7 weeks prior to the detection of shellfish toxicity. The consistency of the signal-response by SPATT is unique among the monitoring data; toxigenic *Pseudo-nitzschia* cell counts and *Pseudo-nitzschia* relative abundance both dropped to zero on 29 Sept and 12 Oct 2009, and pDA indicated declining toxin levels on those dates; the collection of a discrete sample, which indicated an absence of *Pseudo-nitzschia* (toxigenic species or otherwise) and declining toxin levels on 12 Oct is especially significant since the sentinel shellfish 'went toxic' the following week, jumping from zero to above the regulatory toxin limit within a matter of days (non-detect on 14 Oct 2009; 29 μg g^{-1} on 21 Oct 2009).

While our record for PST is incomplete (e.g., no SPATT-PST data are available for the cell detection incident in Nov 2009), the detection of PST in HP20 field extracts exhibited patterns similar to those demonstrated for DA. Concentrations of PST in HP20 field extracts reflected both the presence of *Alexandrium catenella* and the toxin levels measured by CDPH in mussel samples (Fig. 4).

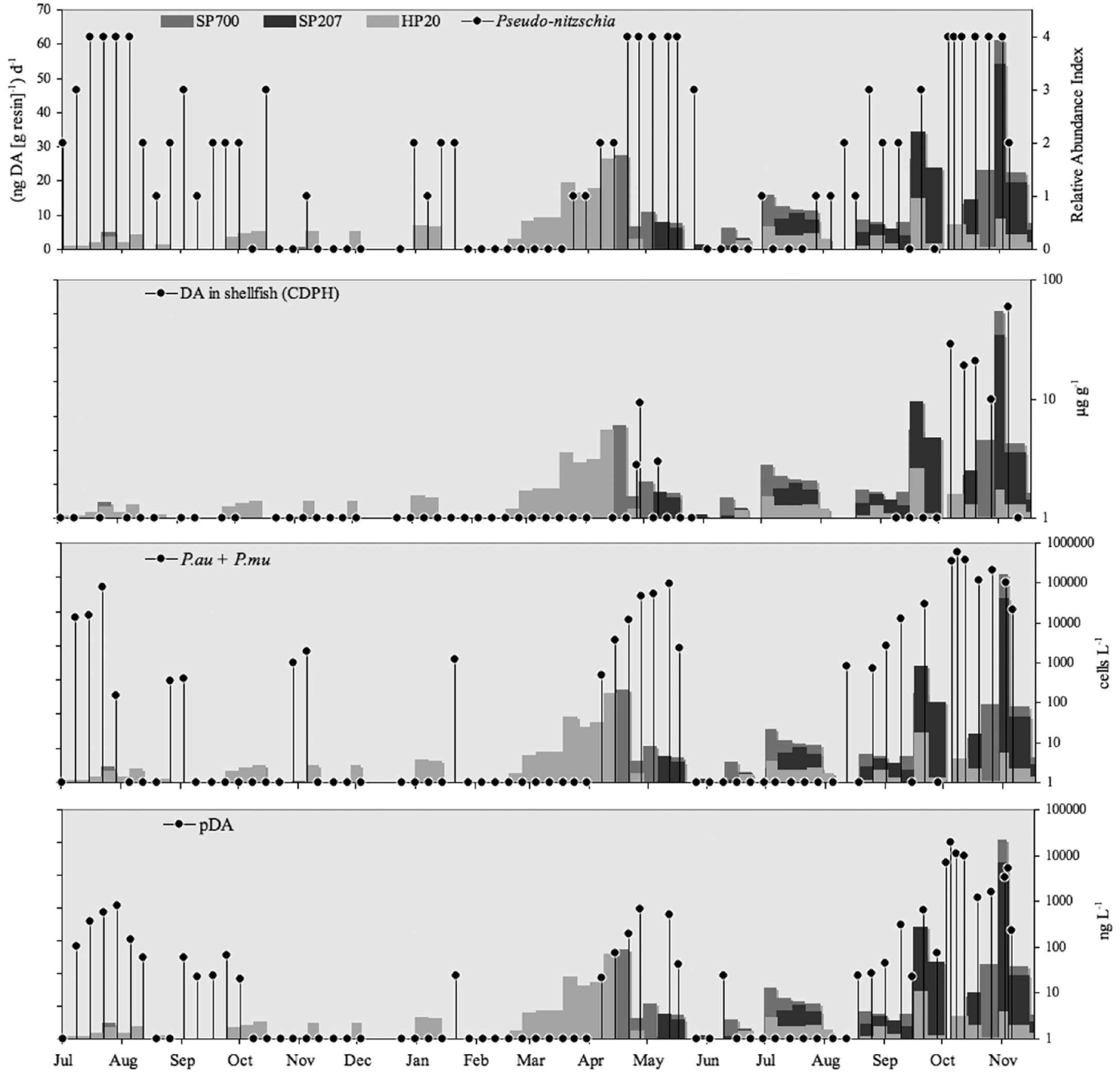


Fig. 3. Results from field deployment of SPATT bags at the Santa Cruz Municipal Wharf from 15 Jul 2008 – 01 Dec 2009. The SPATT field results are overlaid with total *Pseudo-nitzschia* relative abundance (0 = none, 1 = rare, 2 = present, 3 = common, 4 = abundant) (A), domoic acid in adjacent sentinel shellfish, as reported by the California Department of Public Health (CDPH) (B), cell abundance of toxigenic *Pseudo-nitzschia* (*P. multiseries* + *P. australis*) (C), particulate domoic acid (pDA) concentration (D).

Discussion

Deployment strategy and cost—Biotoxin monitoring for the protection of human health requires a reliable resource that can be sampled consistently for monitoring purposes. In California, participants in the CDPH Marine Biotoxin Monitoring Program collect samples of sentinel or wild shellfish for delivery to the state lab for analysis; in most years, the majority of biotoxin samples analyzed by CDPH are mussels (e.g., 69% in 2008). Special significance is given to monitoring activities along the outer coast, where toxin detection might afford advance warning to harvesters in more protected areas. The abundance of mussels along the outer coast of California is

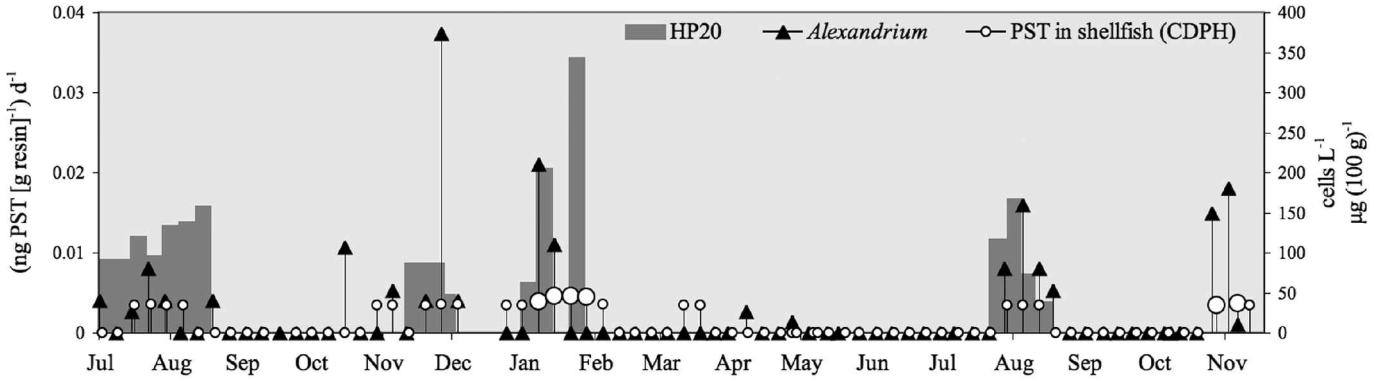


Fig. 4. Results from the analysis of HP20 SPATT field bag extracts for saxitoxin and related paralytic shellfish toxins (PST). All extracts were obtained from HP20 field deployments at the Santa Cruz Municipal Wharf and assayed by Abraxis® ELISA. Also shown are PST in adjacent sentinel shellfish, as reported by the California Department of Public Health (CDPH), and cell concentrations of *Alexandrium catenella*, a known source of PST in the marine environment. Enlarged open circles denote PST measurements by CDPH that were above the limit of detection, nonenlarged open circles denote PST detection through a rapid screening technique with nondetection by analytical means (value shown is the upper bounds, or detection limit) or PST nondetection.

variable, however, both between regions (i.e., northern and southern California) and between sites within a region (Smith 2005). Mussel abundance is subject to change according to various factors including mesoscale oceanographic conditions, biotic and abiotic factors acting on local and broad scales (e.g., predation, tidal patterns), and larval supply and recruitment (c.f. Smith et al. 2009). Within the Monterey Bay region, inconstant mussel abundance has confounded monitoring activities: local monitoring agencies have recently reported low mussel recruitment to supply sites that had been generally reliable, and have expressed difficulty in sustaining and locating adult mussel stock [S. Peters (County of Santa Cruz Environmental Health and Safety) pers. comm.]. While sentinel shellfish availability is inconsistent, the supply of SPATT is controlled only by the working capacity and preference of the monitoring agency. We have successfully deployed SPATT as a passive sampler in a freshwater lake, desalination system feedwater tank, and in a flow-through surface water sampling stream aboard a research vessel (data not shown). Given this flexibility, we anticipate that the primary impediments to widespread use of SPATT are primarily construction and analysis cost and secondarily inter-calibration with mussels or other shellfish.

A comparative cost analysis for shellfish and SPATT is supplied in Table 4. Shellfish costs were estimated according to the extraction procedure and materials described in established protocols (Hess et al. 2005; Quilliam et al. 1995; Quilliam et al. 1998). While the overhead costs estimated for both monitoring programs are comparable (~$200 USD), the programs differ in per-sampler cost. Relative to shellfish, a SPATT bag is lower in cost by 5.5-fold (HP20), 7.8-fold (SP700), and 2.5-fold (SP207). The SP207SS resin is the only case for which the cost of SPATT is higher than for shellfish (1.7-fold). One cost not represented in Table 4 (due to difficulty of quantification) is the cost of time and labor required to generate and maintain a supply of SPATT bags or sentinel shellfish. The construction of 60 SPATT bags, sufficient for over a year of weekly rotations, could be completed within 6 h by a student volunteer. To maintain a sufficient supply of shellfish for deployment at SCMW, shellfish collection, and bagging events occur 2-3 times per year by parties of 2-5 volunteers. While we present this comparison on a line-item basis for purposes of clarity, we do not suggest SPATT as a (less expensive) alternative to sentinel shellfish monitoring. With additional inter-calibration and validation studies, SPATT will most immediately be used to augment ongoing shellfish monitoring practices (Mackenzie 2010). If we reassess the potential of SPATT on a cost-savings basis (i.e., shellfish and SPATT are both sampled weekly, but the shellfish sample is extracted only when DA is detected in the SPATT extract), this screening approach within our field trial would have afforded 2% and 26% cost savings from HP20 and SP700, respectively. This is an internal cost-savings estimate; the implementation of SPATT to 'screen' for shellfish toxicity in a semi-quantitative capacity within the current CDPH monitoring design could afford significant cost-savings by reducing both analytical and shipping costs, the latter of which is substantial component of the CDPH biotoxin monitoring budget.

It should be noted that the analytical and labor costs associated with shellfish biotoxin analysis proved prohibitive for all samples received by CDPH, especially when it became clear that multiple toxins were of concern (PST and DA, versus PST alone). Phytoplankton monitoring was integrated into the CDPH Marine Biotoxin Monitoring Program in 1993 so that shellfish samples could be analyzed on the basis of risk probability; as such, only a fraction of the shellfish samples received by CDPH are actually analyzed (e.g., 26% in 2008). Extraction of a field SPATT sample is comparatively lower in time and labor requirements. The SPATT extraction described here includes a three-step series of column elutions that can be

Table 4. Comparative cost analysis of the supplies and equipment required for domoic acid monitoring by sentinel shellfish and by SPATT. Materials (supplies and equipment) required for sentinel shellfish are according to established protocols (Hess et al. 2005; Quilliam et al. 1995; Quilliam et al. 1998). All costs are in 2010 USD ($). Costs included in "Construction & extraction" are for nonrecoverable supplies and expenses pro-rated for weekly sampling (e.g., weekly shellfish sampling includes $41.20 for the collection permit distributed over 52 weeks, plus the weekly cost of an autovial filter and SPE column). In this study, SPATT and shellfish were both analyzed for DA by LC-MS; the analytical cost for the two monitoring methods was therefore equivalent. Reagents are listed but excluded from the cost estimate due to cost variability, whereas personnel costs are not included and assumed to be equivalent for the two methods.

	Sentinel shellfish		**SPATT**	
Sampler construction	Field collections of shellfish, California Department of Fish & Game annual sport fishing license	41.20	HP20 (3 g) Sorbent, HP20-01	1.00
			SP700 (3 g) Sorbent, SP700-05L	0.60
			SP207 (3 g) Sorbent, SP207-01	2.50
			SP207SS (3 g) Sorbent, SP207SS-01	11.90
			Nitex (100 µm, per bag) Wildco, 24-C34	0.30
			Nitex (53 µm, per bag) Wildco, 24-C27	0.40
Extraction supplies (non-regenerative)	Autovial filter, Whatman Inc., AV125UNAO	2.90	—	—
	SPE column, Supelclean LC-SAX, 57-017	3.40	—	—
Construction & extraction (cost per sample)		**7.10**	**HP20**	**1.30**
			SP700	**0.90**
			SP207	**2.80**
			SP207SS	**12.30**
Specialized equipment	Commercial blender, Waring, 7011-G	211.50	Bag sealer (8"), Clamco, 210-21E	110.00
			Polypropylene columns (50), Bio-rad, 732-1010	104.50
Overhead (total cost)		**211.50**		**214.50**
Shipping (UCSC to CDPH)	**FedEx Priority Overnight**	**33.54**	**FedEx 2Day**	**16.39**
Processing time (per sample)	**Shuck, homogenize, extract & SAX-prep**	**3 h**	**Rinse & extract**	**0.5 h**
Reagents	Methanol, H_2O, Acetonitrile, Citric acid buffer, Formic acid		Methanol, H_2O, Ammonium acetate	

completed within 10-30 min, yielding extracts that are available for immediate analysis of a single toxin or of multiple toxins, as demonstrated (e.g., DA or DA plus PST), We note that others have already demonstrated the ability to use HP20 for a suite of marine toxins (Fux et al. 2008, 2009; Mackenzie et al. 2004; Pizarro et al. 2008a, 2008b; Rundberget et al. 2007, 2009; Takahashi et al. 2007). The resin and extract are reasonably stable when stored at –80°C, providing the ability to archive samples for further analysis at a later time. An evaluation of variability among individual SPATT bags and individual sentinel mussels further demonstrates the advantage of SPATT as an artificial sampling device: extractions of replicate field-deployed SPATT bags demonstrated significantly less variability than extractions of individual sentinel mussels that had been collected from the same deployment bag (14.9-36.9% versus 40.6%).

For some applications, such as ecological monitoring of toxin levels separate from shellfish, or for ecophysiological studies of harmful algae, direct comparison between shellfish and SPATT may not be necessary. For regulatory monitoring purposes, however, SPATT alone would not be acceptable since it does not conform to AOAC guidelines (AOAC International

2000; AOAC International 2006). SPATT may still be useful as an augmentation to regulatory monitoring of shellfish, similar to the phytoplankton observations, in that it can indicate the potential presence of biotoxins in the environment without or in addition to analysis of shellfish. Further inter-calibration, potentially on a site-by-site basis, would likely be required before SPATT could be fully integrated into existing monitoring programs (Mackenzie 2010). This present study supports the inclusion of hydrophilic toxins in future inter-calibration studies and provides evidence of their potential to augment regulatory practices worldwide, including within the United States.

Detection and early warning of a DA toxin event—Observations from a previous DA event along the California coastline established that the monitoring of shellfish alone is not always sufficient for adequate warning of food web contamination at levels that are threatening to marine mammal or human populations (Scholin et al. 2000). This previous DA event, triggered by a bloom of *Pseudo-nitzschia australis* in May and June 1998, resulted in the death of over 400 sea lions; although DA was detected in phytoplankton, northern anchovy (*Engraulis mordax*), and California sea lion (*Zalophus californianus*) samples, concurrent samples of blue mussels (*Mytilus edulis*) contained little to no DA (Scholin et al. 2000). The converse can also occur: DA-event observations of *M. edulis* populations in Cardigan Bay, Canada, reached toxin levels higher than could be explained by a single-compartment uptake-clearance model of DA flux (> 300 ppm), suggesting that biological factors (increased grazing or the suppression of metabolic efficiency, or both, under bloom conditions) complicate the relationship between the amount of DA available in particulate form in the water column and the amount that accumulates in mussels (Silvert and Rao 1992). Both accretion and depuration vary according to seawater temperature and mussel size, with kinetics driven largely by the digestive gland (Blanco et al. 2006; Novaczek et al. 1991). The efficiency at which DA is accumulated in *M. edulis* is 1% to 5% (Wohlgeschaffen 1991); the rate of depuration, originally estimated at 17% d^{-1}, has been recently corrected to 87% d^{-1} (Krogstad et al. 2009).

Synthetic passive sampling devices can be represented by a one-compartment sampling model (e.g., Fig. 1 in Stuer-Lauridsen 2005), with uptake according to chemical potential gradients. These chemical potential gradients can be affected by environmental conditions (flow, temperature, biofouling, etc.), and permeability reference compounds have been recommended as a means to quantify and correct for these factors (Booij et al. 1998; Huckins et al. 1996; Stuer-Lauridsen 2005). With the exception of semi-permeable membrane devices applied in conjunction with calibrated reference compounds, passive sampling devices are assessed semi-quantitatively, e.g., for the early-warning detection of toxin incidence or increase (Stuer-Lauridsen 2005).

Our field deployments of HP20 SPATT successfully signaled two shellfish toxicity events 3 and 7 weeks prior to bloom onset (toxigenic *Pseudo-nitzschia* > 10,000 cells L^{-1}), and 7 and 8 weeks prior to the detection of shellfish toxicity; unlike traditional metrics, enhanced DA-signaling by SPATT consistently indicated the development of toxigenic blooms and impending incidents of shellfish intoxication (i.e., in the fall bloom, toxigenic *Pseudo-nitzschia* cell counts and *Pseudo-nitzschia* relative abundance both fell to zero the week prior to bloom onset and pDA declined the week prior to DA detection in shellfish). Sentinel shellfish samples collected and analyzed as part of the CDPH Marine Biotoxin Monitoring Program during the spring bloom period never exceeded 9.2 μg g^{-1} tissue (the regulatory closure limit is 20 μg g^{-1}), and were not toxic above the CDPH detection limit until 8 weeks after DA detection with SPATT (Fig. 3). Unlike the spring bloom, mussel toxicity did exceed the regulatory closure limit during the toxigenic bloom event of fall 2009. This exceedance forced a regulatory reaction from CDPH: the annual mussel quarantine, lifted by CDPH according to schedule on 31 Oct 2009, had to be reinstated 2 weeks later through an emergency statewide press release (13 Nov 2009). Mussel toxicity was measured at its highest level on 20 Nov 2009 (59 μg g^{-1}); SPATT-DA signaling was unprecedented (high) from the deployment rotation immediately preceding that measurement (e.g., SP700; 60 ng DA g^{-1} d^{-1}). Monitoring by SPATT successfully signaled the impending toxigenic blooms of spring and fall 2009, and tracked the unexpectedly sustained toxigenic bloom conditions of fall 2009. This demonstration of SPATT DA-signaling relative to shellfish toxicity and CDPH regulatory behavior indicates its potential to facilitate a more anticipatory, less reactionary, management perspective.

Detection of DA under 'non-event' circumstances—Acute, fatal, or chronic sublethal exposure to DA is increasingly recognized as an emerging threat to both human and wildlife health (Goldstein et al. 2008; Grattan et al. 2007; Kreuder et al. 2005; Ramsdell and Zabka 2008). Data from long-term disease surveillance suggests that DA exposure may be one factor contributing to mortality and failure of population recovery of southern sea otters, a federally listed threatened species (M. A. Miller [CDFG], pers. comm.). Logistic regression models developed for toxigenic *Pseudo-nitzschia* blooms in Monterey Bay have been used to indicate the extent of potential exposure by signaling the incidence of toxigenic bloom events (Lane et al. 2009), but cannot directly address toxin incidence since toxigenic blooms can vary widely in toxicity (Anderson et al. 2006, 2009; Blum et al. 2006; Lane et al. 2009; Marchetti et al. 2004; Trainer et al. 2002). Irrespective of bloom prevalence or toxicity, SPATT affords the direct detection of DA, PST (including STX), and other phycotoxins in the water column and, as an integrative sampling tool, SPATT has the potential to signal the incidence of toxin between discrete sampling events. Observational data from SPATT deployments, applied in conjunction with toxigenic *Pseudo-nitzschia* bloom models or traditional sampling methods (phytoplankton identification,

sentinel shellfish monitoring, etc.), can help to interpret the extent to which the models describe the frequency of toxin exposure (not simply the potential for toxigenic blooms). In this context, integrative toxin detection would be especially valuable across periods of low or transitory toxin incidence, i.e., across periods when modeled bloom predictions might otherwise be (errantly) categorized as 'false positive' due to artifacts of discrete sampling.

As a time-integrative sampling tool, SPATT is designed to detect toxin at levels that would otherwise elude detection. In our field study, SPATT deployments of HP20, SP700, and SP207 signal the presence of DA over periods in which resident toxigenic *Pseudo-nitzschia* species were not detected by weekly phytoplankton observation. Concentrations of pDA tracked closely with toxigenic *Pseudo-nitzschia* abundance; this may, in part, be an artifact of their simultaneous measurement from the same discrete water sample. The patterns of DA-signaling by SPATT, specifically the detection of DA in advance of an impending bloom and intermittent low-level DA-signaling throughout the year, suggest that SPATT deployments resolve the incidence of DA when toxigenic species observation and pDA quantification otherwise fail as indicators. This is not to suggest that SPATT is designed to compete with these other more traditional measures; the semi-quantitative, temporally integrated measurement of DA in the dissolved component of the water is unique in subject, purpose, and, as such, in its potential to reveal otherwise unrecognized patterns of DA presence and absence. Rather, we envision SPATT as "a supplementary technique," which could help to reduce costs associated with traditional shellfish and plankton monitoring methods (Mackenzie 2010) while providing a time-integrated toxin monitoring record, which is useful for regulatory purposes and in other contexts (e.g., toxin model development and validation).

Selection of a SPATT resin—Other solid-phase extraction techniques exist for the extraction of DA from natural media such as seawater and urine (Chan et al. 2007; Piletska et al. 2008). These previously described methods require specialized adsorption media, sample pre-treatment (usually pH adjustment), or both, such that their application toward in-situ adsorption of DA is not feasible (and is not the purpose for which they were designed). The four SPATT resins evaluated exhibit different adsorption characteristics with DA (Table 3) and should be selectively applied according to the goal for which they are implemented.

The HP20 resin is characterized by relatively weak adsorption behavior with DA. We have demonstrated the successful application of HP20 for detection of the hydrophilic toxins DA and PST in the field, and previous studies have demonstrated its applicability for lipophilic toxins (Fux et al. 2009; Fux et al. 2008; Mackenzie et al. 2004; Pizarro et al. 2008a, 2008b; Rundberget et al. 2007, 2009; Takahashi et al. 2007; Turrell et al. 2007). HP20 demonstrates characteristics that perhaps most closely imitate those of a mussel, i.e., a relatively low rate of accumulation (19% over 7 d) and a relatively higher rate of desorption. Of the resins evaluated here, the nonspecificity and weak adsorption behavior of the HP20 suggest that it would most accurately imitate the adsorption and depuration response of a sentinel mussel while affording the constancy of a nonbiological passive sampler. The use of HP20 offers the added benefit that it can be applied toward simultaneous detection of both hydrophilic and lipophilic toxins (Table 1).

The SP700 resin is characterized by moderate adsorptive behavior with DA (Fig. 2). Deployment of SP700 by the Fisheries Research Services (FRS) Marine Laboratory are as nonactivated resin. The activation of SP700 augmented its adsorptive behavior, reducing extraction efficiency, and thereby suppressing DA-signaling. Field deployments of activated SP700 detected DA only twice (05–11 Aug and 12–18 Nov 2008; 4.7 and 0.3 ng DA g^{-1} d^{-1}, respectively). The first deployment of SP700 as a nonactivated resin yielded a significant DA signal (29 Apr–05 May 2009; 27 ng DA g^{-1} d^{-1}). Nonactivated deployments of SP700 tracked declining DA signals through a post-DA-event period when HP20 DA signals fell to undetectable levels; these results are consistent with the characterization of HP20 as a 'leakier' resin and SP700 as a more aggressive adsorptive medium.

The SP207 resin was the most responsive resin in terms of its adsorptive behavior with DA. SP207 field deployments did not begin until 20 May 2009, and its deployment record is therefore limited in coverage. Throughout the period of SP207 deployments, the pattern of DA-signaling from SP207 agrees well with the pattern described by concurrent SP700 resin deployments. The highly responsive adsorption behavior of SP207 (Fig. 2) suggest that this resin may be most suitable for applications where fast adsorption kinetics would be a requirement (e.g., in a flow-through column, or as an adsorptive on a glider or autonomous vehicle).

The deployment of SP207SS for SPATT is the most expensive of the deployment options evaluated in this study, and SP207SS was not evaluated as a field-deployed SPATT resin. In lab trials, SP207SS SPATT bags exhibited the same characteristics as those described for SP207; this is not unexpected, since SP207SS and SP207 differ only in that SP207SS is of smaller particle size (75-150 μm versus > 250 μm). SP207SS was selected for evaluation to assess whether there is an advantage gained from use of the finer-particulate version of SP207 resin type. Based on the adsorption and extraction profiles, adsorption speed and extraction efficiency are not improved in the relatively more expensive SP207SS resin.

In the creation of an improved extraction protocol, our priorities were (1) a low volume requirement for necessary solvents, (2) the use of solvents and chemicals, which would not interfere with analysis by LC-MS, (3) safe and easily disposed of reagents, and (4) the extraction of a whole (un-cut) SPATT bag. For our purposes, the application of the UCSC field protocol both satisfied these priorities and proved sufficient: we

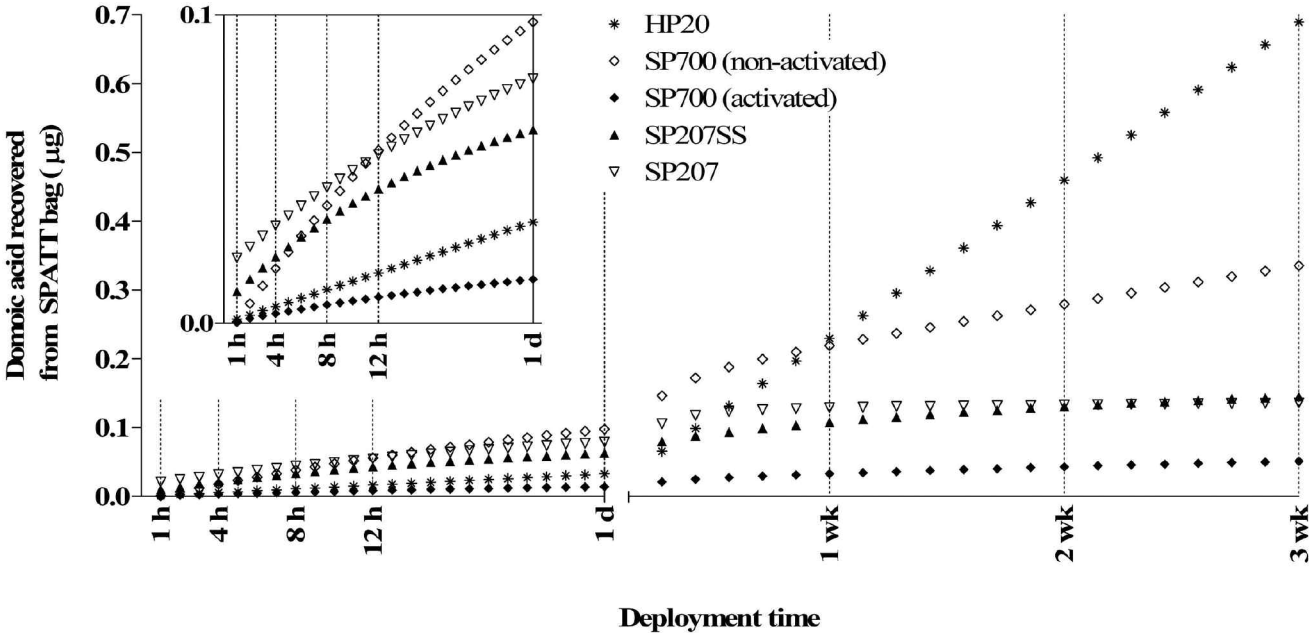


Fig. 5. A graph of hypothetical domoic acid (DA) recovery from SPATT bags of resin types evaluated in this study. The figure utilizes the experimentally determined adsorption and extraction efficiencies, converted to the theoretical amount of DA recovered from SPATT bags of each resin type as a function of the duration of deployment. We assumed a closed system (1000 L) with an ambient dissolved DA concentration of 0.01 nM.

were able to observe DA-signaling from our field-deployed SPATT bags, and the DA-signaling from the various resin types appeared to augment (rather than contradict) discrete observations of pDA, toxigenic and *Pseudo-nitzschia* abundance, *Alexandrium catenella* abundance, and measurements of PST and DA in shellfish.

The adsorption behavior of each resin was representative of its relative extractability, i.e., faster adsorption translates to reduced (or more difficult) extraction. As such, the relative extraction efficiency of each resin must be considered in conjunction with its adsorption profile for proper identification of a resin that is 'optimal' for any specific application. Figure 5 depicts the combination of adsorption and extraction efficiency for each resin to demonstrate the amount of DA that would be recovered from SPATT bags using our extraction protocols, presented as a function of the hypothetical deployment period. While these results are theoretical, according to Fig. 5, deployment periods of 1 week would result in DA-signaling from all three resin types at roughly the same magnitude; slight increases in the DA-signal magnitudes from SP700 and SP207 may be owed to their greater capacity to adsorb DA during transient exposure to toxin. Fig. 5 also highlights the relative advantages of highly responsive resins such as SP207 for deployment in flow-through or mapping applications, and the benefits of using HP20 for prolonged deployments, despite its 'leaky' characteristics.

Much higher recoveries were obtained in nonactivated SP700 laboratory trials when using either an HPLC column (82%) or successive room-temperature soak/transfers with MeOH (69% to 72%), while extraction of HP20 exceeded 99% when resin was removed from the SPATT bag and the Milli-Q rinses were included in the analysis. Milli-Q rinses did not extract DA from the other (non-HP20) resins. While it is important to demonstrate that maximal extraction efficiencies can be obtained, the time, effort, and cost required for achievement of those levels of efficiency detract from the utility of SPATT as a quick, efficient, and inexpensive new monitoring technology – without providing benefit beyond what larger resin bag deployments would provide (i.e., > 3 g dry weight resin). However, since the toxins appear to be stable when the resin is stored at –80°C, the end user always has the option of using our quick extraction protocol followed by more extensive (complete) extraction at a later time.

Comments and recommendations

A primary challenge in this method development was the identification of a reasonable and satisfactory extraction protocol. Just as we adapted an original extraction protocol for our purposes, we encourage further adaptation of our protocol (or the original protocol) for the improvement of extraction efficiency, the minimization of solvent volumes, and/or additional convenience. The resins performed adequately for the purpose of our SPATT-DA field monitoring; this study, therefore, represents the successful expansion of SPATT technology (1) into U.S. waters, and (2) toward detection of DA, a hydrophilic phycotoxin (and in the case of HP20, simultaneous detection of STX and related PSTs). Whereas this is a demonstration of success, it is also a demonstration of broader

potential. The promise demonstrated here for SPATT technology warrants the future development and improvement of protocols allowing for expansion into a more diverse range of applications.

While not attempted as part of this study, we would particularly encourage investigation into the potential regeneration of SPATT resin for re-use. Synthetic adsorptive resins are relatively robust in structure and material, and are routinely regenerated as part of their application in an exceptionally wide variety of contexts. The development of a regenerative capacity would require: (1) the development of an extraction protocol that consistently provides 100% extraction efficiency, (2) a cleaning protocol to eliminate biofouling, and (3) a controlled assessment of resin resilience and performance through successive regeneration cycles. This would further reduce the expense and time associated with SPATT monitoring and would diminish the waste resin produced as a result of its application; these advantages would need to be balanced against the production of additional waste solvents generated as part of the recycling process as well as any loss of performance in the recycled resin.

We illustrate that passive sampling by SPATT is a powerful semi-quantitative tool that can afford new and unique insight into the distribution and prevalence of the hydrophilic biotoxins domoic acid and saxitoxin in the marine environment. Since the use of HP20 and SP700 with lipophilic toxins has been established, this work broadens their potential applicability toward the full range of phycotoxins currently monitored in sentinel shellfish. As such, SPATT is a technology that may support a more holistic regulatory approach, since its relevancy encompasses both lipophilic toxin exposure (e.g., neurotoxic shellfish poisoning, diarrhetic shellfish poisoning) and the hydrophilic toxins addressed in this study (amnesic shellfish poisoning, paralytic shellfish poisoning). Further, signaling by SPATT-DA in advance of shellfish toxicity may allow agencies to assume a more proactive (less reactive) regulatory stance. As an artificial sampling device, SPATT lacks the biological variability and analytical complications inherent to sentinel shellfish, and can be deployed in environments not conducive to sentinel organism monitoring. Since lipophilic and hydrophilic toxins are encountered and addressed by regulatory agencies and research institutes worldwide and within a range of environments, SPATT may afford more efficient and effective regulatory action, cost-savings, and enhanced toxin detection across an equally broad range of contexts. While its potential is apparent, SPATT should be considered complimentary to quantitative observational methodologies until properly standardized to meet the reporting needs of regulatory agencies.

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