Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007

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Abstract

We assess the application of the second-generation Environmental Sample Processor (ESP) for the detection of harmful algal bloom (HAB) species in field and laboratory settings using two molecular probe techniques: a sandwich hybridization assay (SHA) and fluorescent in situ hybridization (FISH). During spring 2006, the first time this new instrument was deployed, the ESP successfully automated application of DNA probe arrays for various HAB species and other planktonic taxa, but non-specific background binding on the SHA probe array support made results interpretation problematic. Following 2006, the DNA array support membrane that we were using was replaced with a different membrane, and the SHA chemistry was adjusted. The sensitivity and dynamic range of these modifications were assessed using 96-well plate and ESP array SHA formats for several HAB species found commonly in Monterey Bay over a range of concentrations; responses were significantly correlated (p < 0.01). Modified arrays were deployed in 2007. Compared to 2006, probe arrays showed improved signal:noise, and remote detection of various HAB species was demonstrated. We confirmed that the ESP and affiliated assays can detect HAB populations at levels below those posing human health concerns, and results can be related to prevailing environmental conditions in near real-time.

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Introduction

Harmful algal bloom (HAB) species include representatives from a number of planktonic taxa that are associated with negative impacts for wildlife (e.g., Trainer et al. 2000; Scholin et al. 2000; Landsberg 2002), human health, and local economies (e.g., Prakash et al. 1971; Steidinger and Baden 1984; Kirkpatrick et al. 2004). While considerable progress has been made toward understanding the bloom dynamics of HAB species through ship-based surveys, much of that work has entailed substantial post-sampling laboratory analyses that are time and labor-intensive. Improving early detection has therefore been identified as a priority for HAB research and management, as well as ocean monitoring in general (HARRNESS 2005; U.S. Commission on Ocean Policy 2004; NSTC Joint Subcommittee on Ocean Science and Technology 2007). This priority has catalyzed the development of a number of field-deployable systems for the detection and monitoring of various phytoplankton and HAB species (e.g., Babin et al. 2005; See et al. 2005; Casper et al. 2007; Duy and Connell 2007). Some examples include imaging flow systems (Sieracki et al. 2005;
under a 14:10 light:dark cycle at 15°C in 0.2 μm filtered /2 medium (Guillard and Ryther 1962) using water from Monterey Bay mooring M1 (36.75 latitude, –122.3 longitude, 34‰). Heterosigma akashiwo (clone CAWR04; Tyrrell et al. 2001) was grown at 18°C, all other conditions were as above. Pseudo-nitzschia spp. enumeration is described in Greenfield et al. (2006). A. catenella was counted by sampling five replicate 1.8 mL aliquots of log-phase culture, staining aliquots with 1 drop of acid Lugol's iodine solution, and enumerating cells by light microscopy using a Sedgewick Rafter chamber. H. akashiwo was enumerated according to M. Adachi (unpubl. data) as follows. One hundred microliters of log-phase culture were added to triplicate 3.9 mL 0.2 μm filtered sea water (FSW) aliquots for a 1:40 dilution. To kill cells without rupturing their cell membranes, diluted culture was heated to 45°C in a water bath for ~3 min to stop cell motility then placed immediately on ice for ~2 min. Aliquots (n = 3, 1 mL each) from each of the three tubes were subsequently counted under a light microscope using a Sedgewick Rafter chamber for a total of 9 discrete counts. To collect samples for SHA analysis, log-phase culture was concentrated on to 25 mm, 0.65 μm pore size hydrophilic Durapore (Millipore) filters using gentle vacuum (5 mm Hg). Membranes were rolled and placed in cryovials (Nalge Company), sample-side facing in, and capped vials were stored in liquid nitrogen until needed.

Preparation and processing of DNA probe arrays for remote detection of HABs—A detailed description of SHA in the DNA probe array format, as well as methods for array printing and processing are described elsewhere (Greenfield et al. 2006). For the current study, capture probes used for 2006 deployments included Pseudo-nitzschia australis, (auD1; Table 1), Heterosigma akashiwo (Het1), P. multiseries/pseudodelicatissima (muD2), and Alexandrium tamarense/catenella (NA1). For work conducted during 2006, the control probe (AlexComp) spotted on the arrays was diluted 1:1000, 1:2000, 1:4000, and 1:8000 (Greenfield et al. 2006). During 2007 laboratory studies and field deployments, an additional capture probe for P. multiseries (muD1) was included, and only a 1:1000 dilution of the AlexComp control was used.

Normalization of conjugate activity—We discovered that activity of the antibody-HRP conjugate employed in the SHA (hereafter referred to as “conjugate”) was not always consistent between batches, even when prepared and diluted using a consistent protocol (data not shown; after Greenfield et al. 2006). The conjugate catalyzes colorimetric and chemiluminescent reactions that equate to abundance of target molecules. Because one of our goals is to achieve a more quantitative framework, it is essential to ensure that the reactivity of the working solution of this reagent is consistent among synthesis lots. For that reason, we developed a quality control procedure to adjust conjugate reactivity based on a known concentration of a synthetic target sequence referred to as a “linker.” A linker is an oligonucleotide complimentary to both capture and signal probes (see also Goffredi et al. 2006;
Greenfield et al. 2006). To prepare the linker, a stock (1 ng μL⁻¹ in 1xTE) of NA1S/ALXS alt L, henceforth NA1-L (Table 1), was diluted to 25 pg mL⁻¹ in 0.2 μm-filtered 3 M GuSCN lysis buffer within 1 h of use to give a 96-well plate SHA target A₄₅₀ of ~0.7 (Greenfield et al. 2006). For the ESP array SHA format, a stock (1 ng μL⁻¹ in 1xTE) of auD1/PSDS L, henceforth auD1-L (the complement of capture and signal probes for *P. australis*; Table 1), was prepared as above to give a target A₄₅₀ of ~0.3. When all other reaction conditions are as reported previously, overall reactivity of the SHA toward a linker is governed by the activity of the conjugate.

To prepare the conjugate, lyophilized ImmunoPure Peroxidase Conjugated IgG Fraction Mouse Anti-Digoxin (Pierce Biotechnology), was resuspended to a theoretical 0.8 mg mL⁻¹ with distilled water (Sigma) then diluted 1:500 with Guardian™ Peroxidase Conjugate Stabilizer/Diluent/Blocker (S/D/B). Aliquots of that 1:500 reagent batch were compared against a reference batch of the same reagent known to have the desired reactivity using the 96-well plate SHA. The new 1:500 batch of conjugate was then diluted accordingly with S/D/B to match the referenced standard (this generally equated to ~1:700–1:800 for the 96-well plate and ~1:1300–1:2000 for the ESP array SHA. The ESP array conjugate activity is used at a weaker concentration than the 96-well plate format because preliminary experiments have shown that array background (region where no probe spotting occurred) increases when conjugate levels are higher (data not shown). For the spring 2007 ESP deployment and laboratory tests, specific conjugate activities for SHA in the 96-well plate and ESP array formats were 1:750 and 1:1776, respectively.

Transition to new ESP array membrane support and standard curves—Generation of standard curves using both the 96-well plate and ESP array SHA formats followed methods described previously (Greenfield et al. 2006). However, the previous ESP array membrane support, Predator (Pall Corporation), was discontinued as a commercial product at the end of 2006 so a new array support was required. A transition to Optitran BA-S 83 Reinforced Nitrocellulose Membrane (Whatman, Schleicher & Schuell), hereafter referred to as ‘Optitran’, was implemented. The method for printing DNA probes on Optitran was identical to Predator. Application of Optitran-based arrays in the ESP, however, differed slightly from Predator in that the membrane was clamped tight in the
puck, no backing filter was used, and the array was initially treated with Western Blocking Solution (Sigma) before addition of lysate (1 mL, 8 min) and then substituted for wash during array processing (Goffredi et al. 2006; Greenfield et al. 2006). Conjugate activity was determined as above.

To generate standard curves for the 96-well plate SHA format, frozen filters ($n = 5$) containing known cell numbers of target species were thawed to room temperature then immediately lysed and analyzed (Greenfield et al. 2006). *P. australis* lysates were serially diluted in lysis buffer to yield $8 \times 10^3$, $4 \times 10^3$, $2 \times 10^3$, $1 \times 10^3$, 500, 250, and 0 cells $250 \mu$L$^{-1}$ (or ‘cells well$^{-1}$’). We chose similar lysate concentrations as previous experiments (Greenfield et al. 2006) using *P. australis* to maximize consistency for comparisons between the two ESP array membranes and conjugate levels. *P. multiseries* was serially diluted in lysis buffer to $1 \times 10^3$, $5 \times 10^3$, $2.5 \times 10^3$, $12.5 \times 10^3$, $6.25 \times 10^3$, $3.125 \times 10^3$, and 0 cells well$^{-1}$. *A. catenella* was diluted to 500, 250, 125, 63, 31, 16, and 0 cells well$^{-1}$, and *H. akashiwo* was diluted $1 \times 10^4$, $5 \times 10^3$, $2.5 \times 10^3$, $1.25 \times 10^3$, 625, 313, and 0 cells well$^{-1}$.

To generate standard curves using the ESP, sample filters ($n = 3$) were homogenized, lysates were diluted as appropriate, then the lysate was split such that one aliquot was analyzed using the 96-well plate SHA and the other aliquot of that same lysate was analyzed using the ESP array. The muD2 and muD1 capture probes both react with *P. multiseries* (Miller and Scholin 1998; Scholin et al. 1999) so standard curves for both were performed simultaneously using the same cultured samples. For all standard curves, results of the 96-well plate SHA were used to estimate the concentration of target species supplied to the ESP array. Array spot intensity was determined by subtracting the average of four equal-sized background regions surrounding individual capture and control probe spots, then mean ($\pm$ SE) spot intensity per probe was reported for images at a 60 s exposure at $1 \times 1$ CCD camera binning.

**Field sampling scheme**—During spring 2006 and 2007, the ESP was deployed for ~2-3 weeks periods in Monterey Bay, California, next to the CIMT mooring M0 (36.83N, 121.90W, depth ~70 m) such that the ESP sampling inlet remained between 5 and 7 m below the surface. In 2006, Deployment 1 was 16-27 March and Deployment 2 was 10-23 April while our 2007 deployment occurred 17 May-11 June. For all deployments, the ESP remotely automated sample collection and processing of DNA arrays, one DNA array per day, for either HABs, invertebrate larvae, or bacteria. Competitive ELISA (cELISA) arrays for domoic acid (DA) were processed on the same days as HAB arrays. The development and application of the cELISA arrays will be described separately (Doucette et al. in prep.). Only results for the ESP arrays for HABs (HAB arrays) are considered here.

During 2006 Deployment 1, the first in-water deployment of the 2G ESP, HAB arrays were successfully processed on 16 and 19 March, but puck assembly and instrument failures prevented use of subsequent arrays. During 2006 Deployment 2, HAB arrays were successfully processed on all 5 scheduled dates (10, 12, 15, 18, 21 April). During the same deployment, four bacteria arrays (Preston et al. unpubl. data), and five invertebrate arrays (Jones et al. 2008) were also processed.

Concurrent environmental data were obtained from two sources: one conductivity, temperature, depth profiler (CTD; Seabird Electronics Inc.) affixed to the ESP and data from the M0 mooring. ESP CTD data were used during Deployment 1 whereas M0 data are presented for 27 March-23 April. Parameters used for this study included surface water temperature (°C) and salinity (‰).

**In situ ESP sampling and sample archival**—The process by which the ESP collects and processes samples in situ is detailed elsewhere (Goffredi et al. 2006; Roman et al. 2007; Jones et al. 2008). For our study, whole water samples (400 to 1000 mL for HAB arrays) were processed and imaged according to Greenfield et al. (2006) with the following modifications: the conjugate was diluted 1:5000 using S/D/B during 2006 but during 2007, conjugate was normalized to a reference activity as above and wash buffer was replaced by Western Blocking Solution.

Shortly after collecting a water sample for the HAB array, the ESP collected and preserved an additional 50 mL aliquot. Upon retrieval of the ESP, archived samples were quartered and processed for FISH following Greenfield et al. (2006). Archived samples may be stored for up to ~1 mo without loss of signal in a FISH assay (Miller and Scholin 2000). Fluorescein-labeled oligonucleotides used for this study are listed in Table 1.

**Ground truthing ESP generated data**—Results of HAB array and FISH analyses from ESP-archived samples were compared against laboratory versions of the same types of analyses using water samples collected manually near the ESP and, when possible, coincident with instrument sampling. To collect samples manually, we affixed a davit with a hand-operated winch on a Boston whaler; this enabled deployment of a profiling CTD and two 5 L Niskin bottles at approximately 5 m and 7 m depth. Water samples were returned to the laboratory within 1 h then combined into one depth-integrated 10 L total volume. Since our deployments involved three types of DNA probe arrays (bacteria, invertebrate larvae, HABs), one DNA array per day, ground truth sampling dates did not always coincide with dates when the ESP processed HAB arrays.

Manual sample processing proceeded as follows. Upon return to the laboratory, water (up to 1000 mL, sample volume matched ESP sample volume when possible) was concentrated on a 0.65 μm pore size hydrophilic Durapore filter in replicates of 4 or more. Triplicate filters were immediately processed using the 96-well plate SHA (Greenfield et al. 2006; Table 1). Replicate filtered samples were stored in liquid nitrogen until needed. Samples were collected for FISH in 10 mL aliquots ($n = 9-12$), then filtered and processed using a 13 mm filtration manifold as per Miller and Scholin (1998, 2000).

For DA, two samples of 400 mL each were collected on a 0.65 μm pore size hydrophilic Durapore membranes and stored in liquid nitrogen until extracted and analyzed. DA was...
extracted in 10% aqueous MeOH, and toxin measurements were performed using a surface plasmon resonance (SPR) optical biosensor (Biacore Q; Biacore), according to Traynor et al. (2006) with several modifications. Two hundred twenty microliters of sample, standard, or Quality Control (1 ng DA mL⁻¹) were mixed 9:1 (v/v) with a rabbit polyclonal anti-DA antibody diluted in HBS-EP buffer (1:300, v/v; GE Healthcare-Biacore). Each sample/antibody mixture was injected (150 μL at 25 μL min⁻¹) onto a CM5 sensor chip (GE Healthcare-Biacore) containing immobilized DA. Antibody not bound by DA in solution was bound to the chip surface causing a change in refractive index. Quantification was based on a calibration curve prepared using DA certified reference material (Inst. for Marine Biosciences) and ranging from 1 × 10⁻³ to 1 × 10⁴ ng DA mL⁻¹. DA concentrations in each sample were calculated using Biacore Q and GraphPad Prism (v. 4) software.

**Assessment**

Remote detection of HAB species using the ESP: 2006—Based on laboratory trials that used the Predator array support prior to the 2006 field season, *P. australis* was known to exhibit a predictable relationship between cell concentration and probe array response between 2 × 10³ to 6.4 × 10⁴ cells mL⁻¹ lysate (Greenfield et al. 2006). It was also known that the HAB arrays could be used to detect *P. multiseries/pseudodelicatissima*, *A. catenella*, and *H. akashiwo* (e.g., Scholin et al. 2008), but the relationship between probe array reactivity and organism abundance was not well characterized prior to the start of field operations. The raw score for interpreting species presence and relative abundance based on the HAB arrays was taken as signal for a given capture probe minus background, as measured by CCD counts. Signals from capture probes that were not significantly higher than background (ANOVA, single factor, α = 0.05) or that were less than background were recorded as non-detect. Of all the DNA probes on the HAB array, only the *P. australis* assay was considered potentially quantitative given the established standard curve (Greenfield et al. 2006).

During 2006 field trials, surface water temperature exhibited a general warming trend 16 March to 23 April, but ranged between 11°C and 14.6°C (Fig. 1a). Salinity ranged between 22.90‰ and 33.15‰, but was typically ~33‰ with the exception of a rain event during the first week of April (Fig. 1b). Results of HAB arrays using the Predator array support membrane during Deployment 1 showed that signal intensi-

![Fig. 1](image-url)
ties for all four capture probes were below background levels for both HAB array dates (Fig. 2a). During Deployment 2, HAB arrays exhibited a weak positive signal for Het1 (*H. akashiwo*) on 10 April, but negative signals for all other capture probes. On 18 April and 21 April, positive signals were detected for Het1, auD1 (*P. australis*), and muD2 (*P. multiseries/pseudodelicatissima*). The NA1 probe (*A. catenella*) did not return a positive signal on any sampling date.

Trends in detection of HABs using the 96-well plate SHA typically followed trends recorded by the ESP (Figs. 2a, b). These results were based upon a conservative lower limit of detection (LLD) of 0.094 at A450 for lysis buffer only (study range of 0.052–0.094). For example, the 96-well plate SHA produced negative results for the beginning of Deployment 1, coincident with negative HAB arrays. During Deployment 2, a slight positive reaction for Het1 (0.155 OD ± 0.011) was observed on 10 April, and on 18 April, positive signals were observed for auD1, muD2, and Het1, similar to the array format.

Estimates of *P. australis* levels can be drawn from the 96-well plate SHA values using previously reported standard curves (Greenfield et al. 2006). Abundances ranged from below the detection limit (10, 13 April) to a maximum of ~1.3 × 10⁴ cells L⁻¹ on 21 April corresponding to a 96-well plate SHA value of 0.167 OD ± 0.032 SE.

Trends in cell abundances using FISH for samples archived using both the ESP, and field samples collected manually and processed using the filtration manifold approaches were similar during both deployments (Fig. 2c, d). While overall estimates varied between the two FISH approaches by up to a factor of 2, this discrepancy could be explained by estimates of cell density being below the established, reliable lower limit of detection on all sampling dates (Miller and Scholin 1998; Greenfield et al. 2006).

Some discrepancies, however, between ESP-generated results and data obtained from routine water sampling did occur. For example, a positive signal was detected for the probe NA1 on 18 and 21 April using the 96-well plate, but HAB array results were negative. Such observations were not entirely unexpected because each entails a fundamentally different mode of sample acquisition. The ESP draws in water through a single intake at a fixed depth over a period of up to ~1 h, and sample intake depth may vary due to tides and current. Moreover, samples archived for FISH using the ESP are collected ~1 h after preparing the previous sample for a corresponding array. In contrast, manual sample collections were generally done while the ESP was collecting a sample for SHA. The 5 L Niskin bottles were tripped roughly 5 m and 7 m from the surface, and those water samples were combined upon return to the laboratory. The 10 L sample thus represents an integrated water volume from a discrete time frame at two depths. As a consequence, samples collected by the ESP and those collected manually are never directly “matched;” differences in the phytoplankton community structure between the two sampling modes are therefore possible. This problem was
understood, but the only way to obtain truly matched samples was for divers to collect an equivalent volume of water from near the ESP intake over the same time period the instrument was sampling. This was not possible during this study so we had to rely on shipboard collections; thus, potential discrepancies between ESP and manually collected samples were unavoidable. Nevertheless, general trends observed with the instrument can be corroborated qualitatively by returning discrete samples to the laboratory.

Domoic acid (DA) was detected from manual collections on all HAB sampling dates of Deployment 2, but was lowest (4.7 ng mL⁻¹) on 13 April, coincident with negative results on the 96-well plate SHA for *Pseudo-nitzchia* spp. (Fig. 2b,e). DA was highest toward the end of the deployment, and this coincided with HAB array detection of *Pseudo-nitzchia* spp. (Fig. 2a,e).

**Assay performance following transition to new array membrane support and assay chemistry**—Pall Corporation discontinued production of the Predator membrane in 2006, so further field and laboratory studies using that support were terminated. To assess the applicability of the replacement membrane, Optitran BA-S 83, standard curves were generated for both the 96-well plate and ESP array SHA formats using cultured representatives of target HAB species (Fig. 3). Cell concentrations employed reflected ranges that are either at or below levels typically considered threatening given a water sample volume of 400 mL and lysate volume of 2 mL. For example, DA production by *P. australis* and *P. multiseries* generally does not pose health-related or ecological threats at abundances < 2.5 × 10⁴ cells L⁻¹.

![SHA standard curves](image-url)

**Fig. 3.** SHA standard curves using both the 96-well benchtop assay (n = 5 replicate wells ± SE) and HAB array spot intensity (n = 8 array spots ± SE) for *A. catenella* probe NA-1 (A), *P. australis* probe auD1 (B), *P. multiseries/pseudodelicatissima* probe muD2 (C), *P. multiseries* probe muD1 (D), and *H. akashiwo* probe Het1 (E). Note difference in dynamic range (y-axes) among targets.
and < 5.0 × 10^4 cells L⁻¹, respectively (Rhodes 1998; Todd 2003; Greenfield et al. 2006). *A. catenella/tamarense/fundyense* is typically not considered a health threat from a management standpoint at < 1 × 10^3 cells L⁻¹ (Todd 2003; D. Anderson pers. comm.). *H. akashiwo* is often not considered a threat at < 1 × 10^6 cells L⁻¹ (Tyrrell et al. 2002).

The relationships between cell concentrations using both SHA formats were statistically significant and highly correlated (ANOVA, single factor, α = 0.05; Fig. 3). Both assay formats yielded a linear response to an increasing number of target cells as enumerated using microscopy, so direct comparison between the 96-well plate and ESP SHA array formats can be made for matched samples. While the sensitivity and dynamic range differed considerably among DNA probes, the majority of probes enabled detection of cell densities that, within a laboratory setting, are well within ranges of those relevant for human health and wildlife management concerns. Surprisingly, *P. multiseries* probe μD1 exhibited a lower detection level in the HAB array than the 96-well plate SHA (Fig. 3d). Historically, μD1 is considered less sensitive to μD2, which targets both a weakly reacting probe in both SHA and FISH formats relative to P. multiseries. μD1 was not detected by μD1 using the 96-well plate, but was detectable in the HAB array. In contrast, such low concentrations were detected in both SHA formats by μD2. These results indicate that false-positive signals for some *Pseudo-nitzschia* spp. blooms and suggests that further assay refinement may be necessary. This also reinforces the need characterize performance of the ESP empirically and establish how the detection method functions in a variety of situations.

**Table 2.** Matrix of cross-reactivity observed while generating standard curves using ESP arrays

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<th>Capture probe on ESP array</th>
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<td>NA-1</td>
<td>+</td>
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<td>Het1</td>
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*Capture probe on ESP array refers to probe that exhibited cross-reactivity and lysate target refers to capture probe corresponding to species homogenate. +++ = cross reactivity observed at top three cellular lysate concentrations, no cross reactivity observed at lower concentrations; ++ = cross reactivity only observed at highest two cellular lysate concentrations; + = cross reactivity observed at highest cellular lysate concentrations only; – = no cross reactivity for any cellular lysate concentration; N/A = not applicable.*

Array-to-array variability and reproducibility of the SHA was assessed using *A. catenella* as a representative species. Target lysate concentrations represented high, medium, and low levels as 500, 125, and 31 cells well⁻¹, respectively (Fig. 4). Some array-to-array variability was found, and the 96-well plate format appeared slightly more sensitive to small changes in *A. catenella* densities than did the HAB array. Nonetheless, a strong correlation was found between HAB array spot intensity and cellular lysate concentration (R² = 0.9686; Fig. 4), and this correlation was highly significant (ANOVA, single-factor, α = 0.05, P < 0.001).

Some probe cross-reactivity was observed in the DNA array SHA format when samples had high concentrations of *Pseudo-nitzschia* spp. (Table 2). By comparison, no HAB array cross-reactivity was observed when samples were presented with samples containing high concentrations of *A. catenella* or *H. akashiwo*. In the 96-well plate SHA format, no cross-reactivity was observed for any probe when non-target species were present at the highest concentration used on the corresponding standard curve. These results indicate that false-positive signals for some *Pseudo-nitzschia* spp. could be returned by the ESP during mixed *Pseudo-nitzschia* spp. blooms and suggests that further assay refinement may be necessary. This also reinforces the need to characterize performance of the ESP empirically and establish how the detection methods function in a variety of situations.
given region under normal, field conditions that may give rise to blooms. One intended use of the ESP is as a sentinel for harmful blooms, so positive signals for *Pseudo-nitzschia* spp. could still be interpreted as indicators of the onset of a potentially harmful event, but additional sampling may be required during blooms to verify the presence of individual species.

**Performance of new array membrane support and chemistry in a field setting: 2007**—A primary objective of our field operations during 2007 was to assess the quality of the Optitran support and chemistry modifications. During the 2007 spring deployment, the ESP successfully developed HAB arrays and archived samples for FISH on each of the seven scheduled dates. Additionally, water samples were collected for ground-truthing instrument data on 17, 21, 23, 25, 30 May and 1, 8, 11 June as described above. A CTD and in situ ultraviolet spectrophotometer (ISUS; Johnson and Coletti 2002) attached to the ESP recorded T, S, % light transmission, chlorophyll fluorescence, and nitrate concentrations (NO₃), respectively, at 20 min intervals throughout the deployment. The most notable time period was 25 May and 30 May, when an increase in fluorescence (up to 23.4 mg chl m⁻³) was associated with a decrease in % transmission and a subsequent reduction of NO₃ (Fig. 5). The latter part of the deployment was also characterized by a highly structured water column, as evident by the ESP fluctuating between thin layers. The ESP study site is in an area characterized by intermittent algal blooms, seasonally structured water column, and strong internal wave perturbations (Ryan et al. 2005). The formation of surface layers could therefore have consequences for interpretation of ESP data as the depth of the sampling inlet will vary over a tidal cycle, and the timing of instrument sampling has typically been constant during a deployment.

**Fig. 5.** Results from 2007 field operations depicting the 25 mm diameter HAB array map showing location of capture probes; auD1 = *Pseudo-nitzschia australis*, muD2 = *Pseudo-nitzschia multiseries/pseudodelicatissima*, muD1 = *P. multiseries*, NA-1 = *Alexandrium catenella*, and Het1 = *Heterosigma akashiwo*. The black squares within the array represent control probes that are independent of sample (A). Examples of imaged arrays with location of capture spots outlined (B). Environmental data corresponding to ESP arrays are shown; solid lines represent times when samples from depicted DNA array were collected.
Array membrane and chemistry modifications were highly effective at reducing background thereby improving overall assay sensitivity and performance (Fig. 5). Contrary to 2006, a positive signal was returned for at least one target on all days HAB arrays were developed, and these signals can be used to derive approximate cell densities using the above-described standard curves (Figs. 3, 6a). Examples include *A. catenella* probe NA1 (17 May, ~300 cells L⁻¹), *H. akashiwo* probe Het1 (21 May, ~1.8 × 10⁴ cells L⁻¹), and *P. multiseries/pseudodelicatissima* probe muD2 (4 June, ~9 × 10³ cells L⁻¹). We recognize that these estimates are based upon cultured reference species, and rRNA content may vary according to age or nutritional status (e.g., Anderson et al. 1999; Miller et al. 2004; Smith et al. 1992). Nevertheless, we believe that our estimates are reasonable representations of population changes that were captured by a series of discrete samples. Additionally, the HAB arrays followed trends observed from samples collected manually and processed in the laboratory (Fig. 6b-d). DA analyzed from manually collected samples tended to be highest during the beginning of the deployment (Fig. 6e), which did not correspond with population trends, although an increase in DA was observed on 11 June, coincident with positive signals from probe muD1 (Figs. 5, 6c, d). As in 2006, some discrepancies between analyses of samples collected using the Niskin bottles versus those collected by the ESP were noted.

**Discussion**

We have demonstrated that the 2G ESP can detect targeted HAB species in situ and showed that trends in ESP results are corroborated qualitatively by trends in phytoplankton abundances elucidated by periodic, manual water sampling. The transition from our previous to current ESP array support and chemistry has increased analytical rigor and improved data quality. Such enhancements are necessary as we work toward interpreting field data in a more quantifiable fashion and integrating the ESP into a larger observatory framework. As this contribution represents the first in a series depicting field deployments of the 2G ESP, several issues, particularly those involving sample collection and data interpretation became evident. A primary example is the logistical challenge of quantitatively assessing performance of a deployed ESP when the water column is highly structured. Without divers, obtaining samples that are well matched to those collected by the instrument is problematic when collections must be made from a ship. Despite that limitation, during 2006, we showed that HAB arrays processed in situ produced results that followed trends of natural phytoplankton abundance estimates using laboratory analyses. Dates when positive signals were returned from HAB arrays for target organisms were highly coincident with positive signals derived from water samples that were processed using the 96-well plate SHA. Cell counts of *Pseudo-nitzschia* spp. using the FISH technique also produced trends that followed signal presence and intensity using SHA. These results further validate the instrument’s performance in detecting HAB species in situ.

![Fig. 6. Results from 2007 field operations depicting cell density estimates from analyzed HAB array images at a 60-s camera exposure (A), 96-well plate SHA absorbance values from manually collected field samples (B), and cell counts using fluorescent in situ hybridization from samples collected and processed using a benchtop manifold (C) and ESP archival procedures (D).](image-url)
When comparing cell counts using FISH between sample filtration and archival using the manifold versus the ESP, counts were often greater using samples collected and archived using the instrument. We are not certain why this is occurring, but sample collection differences (integrated versus discrete water volume) were likely contributors. Another explanation could be that in the majority of cases counts were below the LLD using both approaches. In all but one instance (21 April 2006), estimates of abundances of *P. australis* cells were below the lower limit of detection (LLD) determined by Greenfield et al. (2006) for a statistically reliable calculation of cell density using either the filtration manifold (\(-2.5 \times 10^4\) cells L\(^{-1}\)) or a quartered ESP filter \((-7 \times 10^4\) cells L\(^{-1}\)), so cell counts for FISH should be viewed as primarily qualitative in this study. While counts of *P. multiseries/pseudodelicatissima* using the probe muD2 typically returned higher estimates of cell density than did counts using auD1, the LLD of muD2 has not been rigorously assessed for the ESP manifold sample and archival procedure. Miller and Scholin (1998) found the % SE to be > 20% for enumeration of 2.5 \times 10^4 cells L\(^{-1}\) *P. multiseries* diluted in whole seawater using the filtration manifold, which is greater than our LLD for *P. australis* for the same method.

During 2006 field operations, the most notable environmental feature was the strong rain event during the first week of Deployment 2 followed by a steady increase in surface water temperature. Elevated freshwater input has been associated with *Pseudo-nitzschia* blooms previously (e.g., Scholin et al. 2000). However, other environmental factors were not assessed so the extent to which specific environmental conditions affected *Pseudo-nitzschia* spp. presence and DA production in Monterey Bay during 2006 is inconclusive.

There are four key findings associated with the spring 2007 deployment. First, modifications to assay chemistry and array membrane support produced DNA arrays with less background and greater signal (e.g., compare Figs. 1 and 5). Second, we observed changes in HAB array signals of target populations, and we can determine approximate cell densities using 2007 standard curves. Third, population changes can be related to prevailing environmental conditions. Fourth, although this deployment did not coincide with a major bloom of any target species, we proved that remote detection of HAB species at “pre-bloom” concentrations was feasible. These results suggest the ESP and affiliated assays warrant further evaluation as research and monitoring tools. Given the assays available now, there are number of locations in the U.S. and abroad where such tests would be especially informative. These regions include Puget Sound and coastal Washington, California, the Gulf of Mexico, the Gulf of Maine, Japan, and New Zealand. We are now working with groups in those regions to undertake such joint field studies.

**Conclusions and future directions**

Application of the SHA technique and low density DNA probe arrays using the ESP is a promising means of detecting HAB species remotely. The Optitran membrane support and modifications to assay chemistry improved performance of the method considerably. Furthermore, standard curves generated using the new array membrane support and assay chemistry responded quantitatively to target species over ranges that reflect “pre-bloom” concentrations. The methodology for producing custom formulations of expendable reagents, creating standard curves, and validating instrument performance has been established. We are therefore more confident of the accuracy and reproducibility the SHA chemistry, and we see opportunities for using this approach to augment monitoring and resource management programs in the future. Whereas some cross-reactivity was observed in the HAB array, this did not translate to the 96-well plate SHA format suggesting that field verification is still necessary or that additional fine-tuning of these modifications may still be necessary. Finally, the ability to detect DA using the cELISA is now possible (Doucet et al. in prep.).

Future directions should continue to explore the utility of ESP-generated long-term datasets for enhancing our understanding of temporal variability of target species. There are also opportunities to improve field validation. For example, *H. akashiwo* was detected in the majority of water samples using both SHA formats. However, since this organism does not withstand ethanol fixation well (Tyrrell et al. 2001), validation of field samples using our approaches for FISH is not feasible. Consequently, other methods should be considered to evaluate performance of that assay (e.g., O’Halloran et al. 2006). Additional investigation into the ramifications of different modes of sample acquisition (ESP versus ship-based) and data interpretation is needed. The stability of ESP probe arrays and the long-term stability of prongs used during signal validation with the 96-well plate SHA should also be evaluated further.

We believe that the ESP can be embedded within larger observatory networks and applied toward a variety of research and monitoring programs. In the near-term, the instrument could be used to indicate the presence of target species and place them within a suite of physical and chemical parameters on a predefined sampling schedule. However, it is important to bear in mind that even “rapid” molecular analytical techniques by today’s standards are excruciatingly slow compared with data collection rates routinely obtained from commercially available bio-optical, chemical, and physical oceanographic sensors. Starting with a live sample it is possible to obtain molecular analytical results in ~1 h in a well-equipped laboratory. Attaining and sustaining the same performance in situ is challenging to say the least. Given constraints imposed by consumption of reagents and limited power availability, in situ application of sensors like the ESP will likely benefit greatly from an adaptive sampling strategy. For future operations, we envision programming the instrument to sample at an appropriate base-line frequency, then collecting additional samples in response to a hierarchical set of environmental variables relevant to the research or monitoring program at-hand. In pursuit of these objectives, MBARI is currently building additional
copies of the instrument for understanding spatial variability within a given ecosystem, as well as to spur technology transfer.

References


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