

Diversity and toxicity of *Pseudo-nitzschia* species in Monterey Bay: Perspectives from targeted and adaptive sampling

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ABSTRACT

Monterey Bay, California experiences near-annual blooms of *Pseudo-nitzschia* that can affect marine animal health and the economy, including impacts to tourism and commercial/recreational fisheries. One species in particular, *P. australis*, has been implicated in the most toxic of events, however other species within the genus can contribute to widespread variability in community structure and associated toxicity across years. Current monitoring methods are limited in their spatial coverage as well as their ability to capture the full suite of species present, thereby hindering understanding of HAB events and limiting predictive accuracy. An integrated deployment of multiple in situ platforms, some with autonomous adaptive sampling capabilities, occurred during two divergent bloom years in the bay, and uncovered detailed aspects of population and toxicity dynamics. A bloom in 2013 was characterized by spatial differences in *Pseudo-nitzschia* populations, with the low-toxin producer *P. fraudulenta* dominating the inshore community and toxic *P. australis* dominating the offshore community. An exceptionally toxic bloom in 2015 developed as a diverse *Pseudo-nitzschia* community abruptly transitioned into a bloom of highly toxic *P. australis* within the time frame of a week. Increases in cell density and proliferation coincided with strong upwelling of nutrients. High toxicity was driven by silicate limitation of the dense bloom. This temporal shift in species composition mirrored the shift observed further north in the California Current System off Oregon and Washington. The broad scope of sampling and unique platform capabilities employed during these studies revealed important patterns in bloom formation and persistence for *Pseudo-nitzschia*. Results underscore the benefit of expanded biological observing capabilities and targeted sampling methods to capture more comprehensive spatial and temporal scales for studying and predicting future events.

1. Introduction

Members of the diatom genus *Pseudo-nitzschia* (Peragallo), comprising several species with known capacity to produce the excitatory neurotoxin domoic acid (DA; reviewed in Trainer et al., 2012; Lelong et al., 2012), are generally considered to have a cosmopolitan distribution (Hasle, 2002). Bloom initiation, persistence and decline are

associated with a variety of anthropogenic influences (e.g. eutrophication, global changes in water temperatures, shifts in pH) and natural forcings (e.g. advection, upwelling, stratification, grazing, parasitism; for reviews see Lelong et al., 2012; Trainer et al., 2012). Given the transferability of DA throughout marine food webs (e.g. Lefebvre et al., 2002; Kvittek et al., 2008; Trainer et al., 2012), toxic blooms can threaten human (amnesic shellfish poisoning [ASP]; Perl

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et al., 1990; Todd, 1993; Bates et al., 1989, 1998) and marine mammal and bird (domoic acid poisoning [DAP]; Work et al., 1993; Scholin et al., 2000) health, and severely impact local economies through closures of recreational and commercial shellfish harvesting (e.g. Gallacher et al., 2001; Bill et al., 2006; Smith et al., 2006; Trainer et al., 2007; Brown, 2016). Routine monitoring and management efforts are hampered by challenges associated with sampling patchy phytoplankton populations and limitations in morphological species identification based on light microscopy. The study described herein combined high-resolution detection methodologies with a network of platforms for targeted and adaptive sampling to assess *Pseudo-nitzschia* community diversity and toxin dynamics throughout two different bloom scenarios in Monterey Bay (California, USA).

Nearly every year, Monterey Bay experiences periods of *Pseudo-nitzschia* bloom activity, although with varying degrees of toxin-associated impacts. For example, during 2013, very high cell abundances did not coincide with high DA concentrations, while in 2015 elevated cell abundances were associated with high DA levels as well as a period of marine animal strandings and mortalities. The 2015 event was part of an unprecedented bloom of *P. australis* that stretched from Santa Barbara, California to the Aleutian Islands, Alaska and coincided with a regional physical anomaly, the northeast Pacific “warm blob” (McCabe et al., 2016). Localized anomalous chemical conditions leading to silicate depletion contributed to exceptionally high DA production in Monterey Bay *P. australis* populations (Ryan et al., 2017), while blooms north of California were associated with persistent anomalously warm temperatures that expanded the geographic range of toxic *P. australis* (McCabe et al., 2016). The California Dungeness crab fishery was closed for months, and estimated losses were more than \$48 million (Brown, 2016).

The great inter-annual variability in *Pseudo-nitzschia* ecology in Monterey Bay has been revealed by long-term weekly monitoring from wharf sampling in the northern and southern parts of the bay. This effort has traditionally consisted of whole cell and sandwich hybridization probes for *P. australis* and *P. multiseriata/P. pseudodelicatissima* (Miller and Scholin, 1998, 2000), coupled with DA measurements, on samples from the northern part of the bay (SCW) and light microscopy counts of two *Pseudo-nitzschia* size classes (‘seriata’ sized cells; valve width $\geq 3\mu\text{m}$ and ‘delicatissima’ sized cells; valve width $< 3\mu\text{m}$ [Hasle, 1965; Hasle and Syvertsen, 1997]) from the south part of the bay (MW). This sampling framework has uncovered long-term seasonal patterns in *Pseudo-nitzschia* bloom activity related to environmental factors (e.g. Lane et al., 2009); however, this approach lacks sufficient taxonomic resolution to differentiate the full diversity of toxic species occurring in the bay. Furthermore, the methodologies used exclude species once considered non-toxic that have been shown to produce DA and form blooms (e.g. Adams et al., 2000; Orsini et al., 2002; Trainer et al., 2009; Trick et al., 2010), and other species described more recently (e.g. Lundholm et al., 2012; Lim et al., 2013; Li et al., 2017).

The primary focus for studying and monitoring *Pseudo-nitzschia* in Monterey Bay has traditionally been on *P. australis* and *P. multiseriata*, stemming from the early identification of *P. australis* from toxic events and the presence of *P. multiseriata* in bloom assemblages (e.g. Work et al., 1993; Horner et al., 1997; Scholin et al., 2000), and the implication of the latter species in the first documented case of ASP, which occurred in Prince Edward Island, Canada (Bates et al., 1989; Perl et al., 1990; Rao et al., 1998). Early records indicate that *Pseudo-nitzschia* species, and specifically *P. australis*, have been a part of the phytoplankton assemblage within the bay for at least several decades. Bolin and Abbott (1963) reported that the genus ‘*Nitzschia*’ (originally included *Pseudo-nitzschia*) was the fourth largest group counted over a six-year study period. Scanning electron microscopy of siliceous frustule cell walls and culture-based toxicity studies have been used to confirm the presence of *P. australis* in numerous historical events in Monterey Bay (e.g. Hasle, 1972; Buck et al., 1992; Garrison et al., 1992; Villac et al., 1993). While

P. australis has been implicated as the main DA producer in the region, other toxic and non-toxic *Pseudo-nitzschia* species have been identified over the past several decades (e.g. Bigelow and Leslie, 1930; Cupp, 1943; Villac et al., 1993; Walz et al., 1994; Horner et al., 1997; Bates et al., 1998; Lundholm et al., 2006; Lelong et al., 2012 [review]). It has remained unclear how these other species fit into *Pseudo-nitzschia* assemblages within Monterey Bay. The ability to fully understand community structure and succession through bloom initiation, persistence and decline is thereby severely restricted, which then affects downstream modeling and management efforts. It is highly plausible that forecasting models (Anderson et al., 2009, 2011; Lane et al., 2009) have been impacted by the current approach, leading to discrepancies such as overestimation of DA levels in 2013 (C. Anderson, R. Kudela; pers. comm.).

Advancing predictive skills for this important HAB-forming genus requires a more complete description of community composition and DA production, and better sampling of populations. Toward this goal, this study applied mobile and stationary platforms coupled with high-resolution methods for cell and toxin detection. Further, the mobile platforms employed autonomously targeted sampling of bloom patches. These capabilities allowed us to uncover *Pseudo-nitzschia* population structures on a sizeable spatial and concentrated short-term (~1 month) temporal scale during contrasting bloom years in Monterey Bay. This approach yielded new insights into regional bloom dynamics, which can be used to help further understanding of inter-annual variation in *Pseudo-nitzschia* ecology and to advance prediction.

2. Materials and methods

2.1. Sample collection

2.1.1. Wharf sampling

As part of an ongoing long-term monitoring program, samples were collected weekly on the same day from the Santa Cruz (SCW; 36° 57.48' N, 122° 1.02' W) and Monterey Municipal Wharves (MW; 36° 36.22' N, 121° 53.36' W) prior to and throughout the study period (Fig. 1). Whole

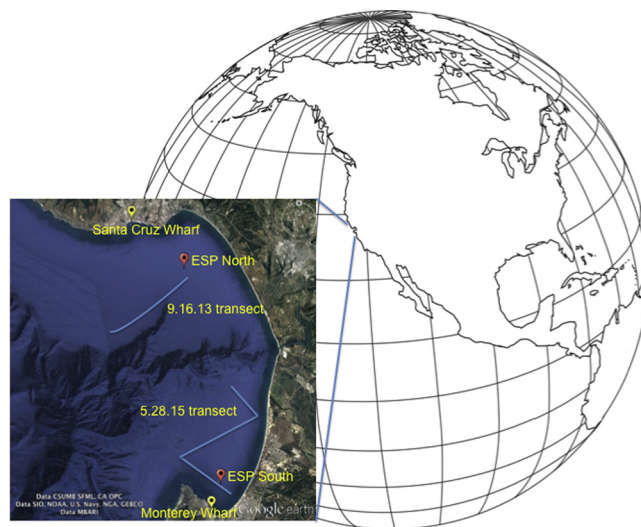


Fig. 1. Sampling sites within Monterey Bay, California (USA) during the two sampling years (2013 and 2015). Integrated whole water samples (to a depth of 5 m) were obtained from Santa Cruz and Monterey Municipal Wharves. Quasi-daily samples were collected and analyzed onboard two moored Environmental Sample Processors: ESP North and ESP South (deployed at same location both years). Cross-bay transects were performed by a Dorado-class AUV with onboard, adaptive sample collection capabilities (the two chosen for further analyses are depicted). Additional samples were collected via boat casts targeting the chlorophyll maximum layer throughout the bay during both sampling years (locations not shown).

water samples from SCW were collected by integration of water samples collected from 3 discrete depths (0, 1.5, and 3 m) with a FieldMaster 1.75l basic water bottle (Wildco, Yulee, Florida, USA). Integrated whole water samples (5, 4, 3, 2, and 1 m) from MW were collected using a 2.2l Van Dorn water sampler (Wildco, Yulee, Florida, USA). Net tows from both locations were obtained using a 20 cm diameter, 20 µm mesh net to concentrate surface waters to a depth of 5 m. Leading up to the start of the 2015 study, surface samples were collected with increased frequency at both wharves. All samples were maintained at ambient temperature and processed within two hours of arriving at the laboratory.

2.1.2. Ship sampling

Monterey Bay was sampled during two different time periods (September 10th to October 7th, 2013 and May 11th to June 5th, 2015) on multiple days via the R/V Rachel Carson and the R/V John Martin as part of a five-year Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) study. Chlorophyll fluorescence was measured with CTD profilers (SeaBird Electronics, Bellevue, Washington, USA) to identify and sample from within and outside chlorophyll maximums using Niskin bottles (10l – R/V Rachel Carson, 5l – R/V John Martin) mounted on a rosette. Samples were processed onboard as outlined below, with the exception of water used for SHA and ARISA, which was stored protected from direct light and processed at the end of the day back in the laboratory.

2.1.3. Dorado AUV sampling

A Dorado-class AUV (Bellingham et al., 2000) was deployed from the R/V Rachel Carson to perform targeted sampling within chlorophyll maximums based on measurements obtained by an onboard fluorometer and autonomous peak-capture algorithm (Zhang et al., 2010, 2012). A sawtooth profiling trajectory mapped vertical water column structure and a water sampling system collected ten 1.8l ‘gulper’ samples per mission (described in Ryan et al., 2010). During the 2013 deployment, there were only nine samples obtained due to malfunction in one sampler. Samples were processed onboard the ship as outlined below, with the exception of water used for ARISA, which was processed in the laboratory at the end of the day.

2.1.4. ESP sampling

Details of the Environmental Sample Processor (ESP) can be found in Roman et al. (2007) and Scholin et al. (2009). The moored instruments were equipped with DNA and protein arrays for near real-time detection of target organisms and associated toxins. For this study, one ESP was deployed in the north part of the bay (36.905°N, 121.936°W) at 7 m depth, and one was deployed in the south part of the bay (36.639°N, 121.879°W) at 5 m depth, from September 10th through October 21st, 2013 and May 10th through June 5th, 2015 (Fig. 1). Both instruments were programmed to collect daily samples, unless a decrease in water temperature (a proxy for upwelling conditions) was detected. Detection of upwelling conditions triggered an unscheduled sampling event, either autonomously by the ESP or through operator intervention. All analyses were performed in situ, as described below.

2.2. Sample processing

2.2.1. FISH

Fluorescence In Situ Hybridization (FISH) was performed on depth-integrated water samples from SCW. The detailed procedure for *P. australis* (auD1), *P. multiseriata* (muD1), and *P. multiseriata/P. pseudodelicatissima* (muD2) probes is outlined in Scholin et al. (1996). Cells were viewed using a Zeiss AxioImager A1 microscope fitted with a fluorescein bandpass filter set (excitation 460–500 nm; emission 510–560 nm) and a 120 W light source (EXFO X-Cite 120). Images were acquired with a Zeiss AxioCam HRC camera.

2.2.2. Phytoplankton counts

Cell counts for *Pseudo-nitzschia* were performed on net tow samples collected from MW. After transport to the laboratory, 0.1 ml of 50% w/v glutaraldehyde was added to 10 ml of net tow material. A Nannoplankton Counting Chamber (PhycoTech, St. Joseph, Michigan, USA) was used to count cells from a 66 µl sample aliquot on an Alexis Scientific microscope through a 10X objective. *Pseudo-nitzschia* cells were classified into two size categories (Hasle, 1965; Hasle and Syvertsen, 1997): the larger ‘seriata’ sized cells (valve width > 3 µm) and the smaller ‘delicatissima’ sized cells (valve width < 3 µm). Total phytoplankton community counting, including the two *Pseudo-nitzschia* size classes, was carried out on gulper samples obtained by the Dorado AUV. Samples were preserved in 1% acidic Lugol’s upon retrieval of the Dorado after a transect mission (approximately 2–4 h). Samples were stored in amber polypropylene bottles (Thermo Scientific, Wilmington, DE, USA) at 4 °C until analysis. A volume of 25 ml was concentrated down to 2.5 ml via gentle centrifugation (1700 g, 15 min), and a volume of 1 ml of material was counted on a Sedgewick Rafter cell as described above.

2.2.3. Particulate domoic acid

Particulate matter from 50 to 250 mL of whole water was concentrated onto a GF/F filter (Whatman, GE Healthcare BioSciences, Pittsburgh, PA, USA). Filters were placed into cryovials and stored at –80 °C until analysis, while shipboard samples were first placed in liquid nitrogen, then subsequently stored at –80 °C. Filters were extracted in 3 ml of 10% methanol (prepared in ultrapure water) for particulate DA analysis. All extracts were sonicated for 30 s at a level of approximately 10 W (RMS), 0.2 µm filtered (Millex, Millipore, Billerica, MA, USA), and cleaned following a solid phase extraction procedure (Wang et al., 2007). Cleaned extracts were stored at 4 °C until analysis. Domoic acid analysis was conducted on an Agilent 6130 LC–MS system (Agilent Technologies, Santa Clara, CA, USA) with an Agilent Zorbax Rapid Resolution column. The toxin 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 an 8-point standard curve using a certified DA standard (NRC CRM DA-f).

2.2.4. ARISA

Automated Ribosomal Intergenic Spacer Analysis, a method to determine relative abundances based on a ribosomal target, was performed on samples collected from Dorado gulpers and ship casts. 200–500 ml were low-vacuum (5 mmHg) filtered onto 25 mm diameter, 0.65 µm pore size Durapore® membrane filters (Millipore, Cork, Ireland). Filters were transferred to 2 ml polypropylene cryovials (Nalgene Nunc International, Rochester, NY, USA) with sample side facing inward, snap frozen, and archived in liquid nitrogen or at –80 °C. Environmental DNA samples were prepared for ARISA as outlined in Hubbard et al. (2014). Briefly, genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) and amplified and prepared for ARISA using the *Pseudo-nitzschia*-specific ITS1 primer set PnallF (5'-TCT TCA TTG TGA ATC TGA-3') and PnallR (5'-CTT TAG GTC ATT TGG TT-3') (Hubbard et al., 2008). Purification of PCR products for ARISA was conducted using MultiScreen-PCR₉₆ filter plates (EDM Millipore, Darmstadt, Germany), and 1 ng of product was analyzed on an ABI 3730 XL using a LIZ600 size standard. Electropherogram analysis with DAX software (Van Mierlo Software Consultancy, Eindhoven, Netherlands) used published peak calling criteria and US West Coast species assignments for peaks based on amplicon length (Hubbard et al., 2008, 2014; Smith et al., 2018).

2.2.5. ESP DNA and DA arrays

The preparation of DNA and DA arrays, and protocols conducted onboard the ESP, are outlined in detail elsewhere (Doucette et al., 2009; Greenfield et al., 2006, 2008). For the 2013 deployment, probes for *P. australis* (auD1), *P. multiseriata* (muD1), and *P. multiseriata/P.*

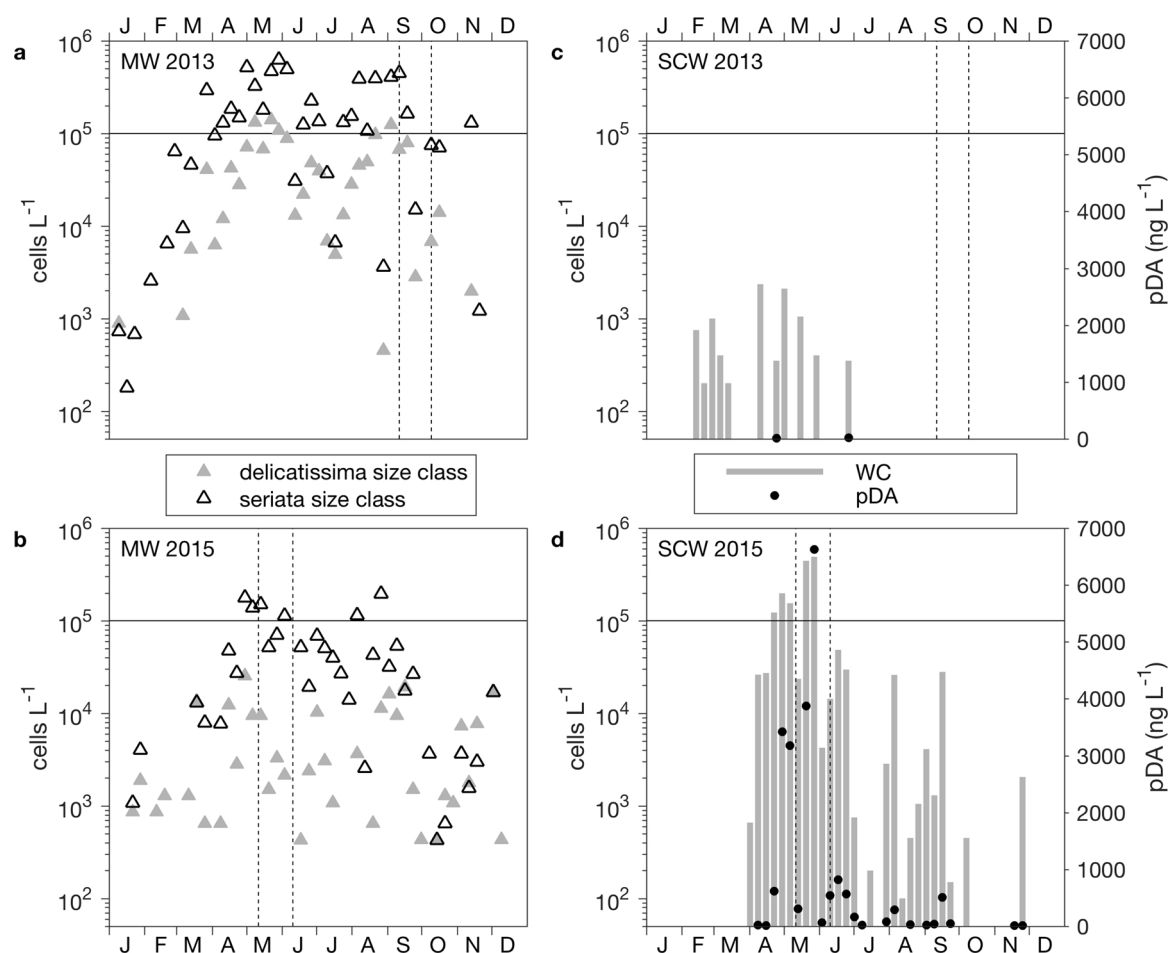


Fig. 2. Weekly time series for *Pseudo-nitzschia* detection at Monterey (MW, south bay) and Santa Cruz (SCW, north bay) Wharves in 2013 and 2015. Cell concentrations at MW (2a, 2b) are based on light microscopy counts of ‘seriata’ and ‘delicatissima’ size classes, while cell abundances at SCW (2c, 2d) are combined results for *P. australis* and *P. multiseriata* whole cell hybridization probes (the latter species was detected only on the following dates in 2015: April 8 [2.55×10^4 cells L^{-1}], April 15 [2.73×10^4 cells L^{-1}], April 22 [1.03×10^5 cells L^{-1}], April 29 [4.85×10^3 cells L^{-1}] and May 6 [1.65×10^3 cells L^{-1}]). Absence of a black dot indicates a negative pDA result for that week. Dashed lines indicate time period for ESP deployments, solid line indicates bloom threshold used for monitoring (Andersen, 1996).

pseudodelicatissima (muD2) were included on DNA arrays (Scholin et al., 1999). For the 2015 deployment, additional probes were added to the arrays (Bowers et al., 2017): *P. arenysensis* (ary1), *P. fraudulenta* (frD2), *P. pungens* (pung1) and an alternative probe for *P. multiseriata* (muD3). Details for preparation of standard curves for determining cell abundances are outlined in Greenfield et al. (2008) and Bowers et al. (2017).

2.2.6. Cultures

Multiple ‘seriata’ and ‘delicatissima’ size *Pseudo-nitzschia* chains were isolated from ship casts, gulper samples, and net tows using separate, sterile disposable pipet tips under a dissecting microscope (SZH10, Olympus, Japan) at 10x magnification. Chains were washed two to three times with medium (0.2 μm -filtered *f/2* medium [Guillard and Ryther, 1962; Guillard, 1975] made with Monterey Bay water amended with 106 μM NaSiO_3) and transferred into individual wells of a 12-well plate (Costar) containing approximately 0.5 ml of sterilized medium. The plates were incubated at 15 °C under a 13:11 h light:dark photoperiod. Successfully isolated cultures were transferred to 25 ml borosilicate glass culture tubes containing fresh medium. When cells reached a dense mid-exponential phase, DNA was extracted from a cell pellet and the large ribosomal subunit was sequenced as described by Bowers et al. (2016). In order to determine cellular particulate DA (pDA), representative species in mid-exponential phase were inoculated into fresh medium in triplicate in a step-wise manner to achieve a final volume of 1 l. Before harvest, cultures were inspected for clumping and

health of cells. Two 50 ml aliquots of each culture replicate were low-vacuum (5 mm Hg) filtered onto 25 mm diameter, 0.65 μm pore size Durapore® membrane filters (Millipore). Filters were transferred to 2 ml polypropylene cryovials (Nalgene Nunc International, Rochester, NY, USA), snap frozen in liquid nitrogen and archived at -80 °C. An aliquot from each flask was preserved with 1% acidic Lugol’s in scintillation vials and stored protected from light until cell counts were performed. For DA analysis, manufacturer’s protocol supplied with the Domoic Acid Test Kit (Mercury Science, Raleigh, NC, USA) was followed. Samples were prepared by adding 1 ml of DI water to cryovials containing filters and sonicating 3×10 s at 30% power on ice (Heat Systems, Farmingdale, NY, USA). Cell counts on replicates were performed using a 1 ml Sedgwick Rafter counting chamber (Pysen SGI Ltd., Kent, UK), with a minimum of three rows and 250 total cells counted.

2.2.7. Benthic sandwich hybridization assays

Sandwich hybridization was performed on samples collected from boat casts and the wharf sites. From whole water, multiple 500 ml volumes were low-vacuum (5 mm Hg) filtered onto 25 mm diameter, 0.65 μm pore size Durapore® membrane filters (Millipore). Filters were transferred to a 2 ml polypropylene cryovial (Nalgene Nunc International, Rochester, NY, USA) with sample side facing inward, snap frozen and archived in liquid nitrogen for downstream sandwich hybridization assays. Details for preparing and running SHA plates are outlined in Harvey et al. (2014) and elsewhere (Scholin et al., 1999;

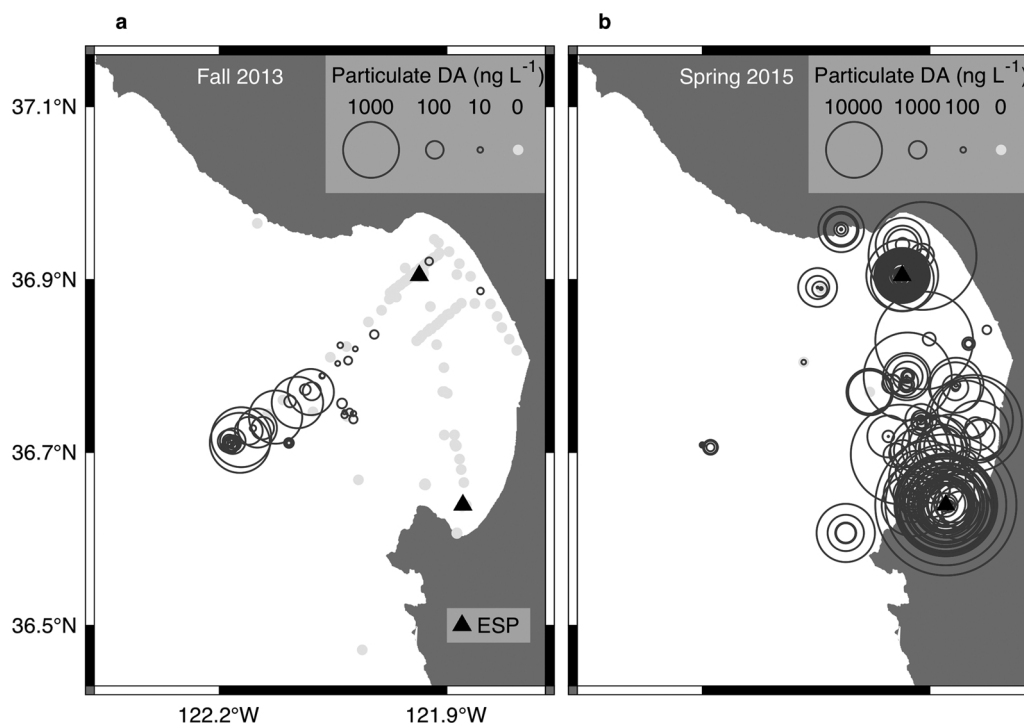


Fig. 3. Particulate DA values for ESP, ship and AUV sampling during the 2013 and 2015 deployment periods. Note scale for 2015 is an order of magnitude greater than for 2013.

Goffredi et al., 2006; Haywood et al., 2007; Marin and Scholin, 2010). Preparation of standard curves for estimating cell abundances is also described elsewhere (Greenfield et al., 2008; Bowers et al., 2017). Sandwich hybridization plates for 2013 samples were prepared with a combination of the following probes: *P. australis* (auD1), *P. fraudulenta* (frD2), *P. multiseriata*/*P. pseudodelicatissima* (muD2) and *P. pungens* (pung1) (Scholin et al., 1999; Bowers et al., 2017). Sandwich hybridization plates for 2015 samples were prepared with a combination of the following probes: *P. arenysensis* (ary1), *P. australis* (auD1), *P. fraudulenta* (frD2), *P. multiseriata* (muD1), *P. multiseriata*/*P. pseudodelicatissima* (muD2, muD3), and *P. pungens* (pung1) (Scholin et al., 1999; Bowers et al., 2017).

3. Results

3.1. Shore station monitoring

Weekly same-day samples from the wharves revealed differences in *Pseudo-nitzschia* abundances and species composition between 2013 and 2015. A key abundance metric is the *Pseudo-nitzschia* bloom threshold used in monitoring programs, 5×10^4 cells l^{-1} (Andersen, 1996). Enumeration of *Pseudo-nitzschia* via microscopy for MW samples (southern bay, Fig. 1) divided populations into the ‘seriata’ and the ‘delicatissima’ size classes, ‘seriata’ being the larger size class containing species with the highest cellular toxin quotas, including *P. australis* and *P. multiseriata*. ‘Seriata’ size class counts in 2013 were above the bloom threshold for 23 weeks and ‘delicatissima’ size class counts were above this threshold for 11 weeks (Fig. 2a). In contrast, 2015 ‘seriata’ size class counts exceeded the bloom threshold for 12 weeks, while ‘delicatissima’ size class counts remained one to two orders of magnitude below the threshold throughout the year (Fig. 2b). Overall, combined counts were 4.6 times higher in 2013 than 2015.

Although *Pseudo-nitzschia* abundances were greater overall in 2013, indicating greater potential for a HAB, a toxic bloom in Monterey Bay instead occurred in 2015. Species composition was different between the two years, with whole cell hybridization results from SCW (northern

bay, Fig. 1) exhibiting a combined average abundance of 3.30×10^4 cells l^{-1} for *P. australis*, *P. multiseriata* and *P. pseudodelicatissima* in 2015, which was more than two orders of magnitude higher than the average of 1.90×10^2 cells l^{-1} in 2013 (Fig. 2c, d). Note, although combined whole cell hybridization data for all species are shown in Fig. 2c and d, values from the muD2 probe (*P. multiseriata*, *P. pseudodelicatissima*) were negligible and contributed to 2015 data only on the following dates: April 8 [2.55×10^4 cells l^{-1}], April 15 [2.73×10^4 cells l^{-1}], April 22 [1.03×10^5 cells l^{-1}], April 29 [4.85×10^3 cells l^{-1}] and May 6 [1.65×10^3 cells l^{-1}].

Consistent with greater abundance of toxigenic species in 2015, pDA was detected more frequently (23 weeks in 2015; 2 weeks in 2013) and showed higher concentrations (10 to 6630 ng l^{-1} in 2015; < 20 ng l^{-1} in 2013). The two highest pDA measurements in 2015 coincided with the two highest cell abundances (Fig. 2d). While the probe results represented combined signal from different species, *P. multiseriata* was only detected in negligible concentrations as outlined above. This indicated dominance of *P. australis* in this bloom, as supported by observations from moored ESPs and AUV targeted sampling described below.

3.2. Targeted and adaptive sampling – pDA and *Pseudo-nitzschia* species throughout the bay

Broader spatial resolution of sampling during the approximate one-month deployment windows reflected the low (2013) and high (2015) pDA values at SCW. In 2013, shipboard bottle casts (surface and DCM [deep chlorophyll maximum]; $n = 104$), Dorado AUV gulper samples (12 transects; $n = 85$) and in situ measurements onboard two ESPs ($n = 29$) revealed zero to trace amounts of pDA within the bay, but identified a toxic (up to 10^3 ng l^{-1}) population concentrated offshore (Fig. 3a). Culturing efforts ($n = \sim 500$ isolates) and SHA performed on a subset of bottle cast samples ($n = 11$; temporal [seven dates] and spatial [four sites]) confirmed that *P. fraudulenta* was the dominant species within the bay, as it was detected in ten of eleven samples (2.06×10^4 to 9.15×10^5 cells l^{-1}), while the remaining probes (*P.*

Table 1

Benchtop SHA was performed on several ship cast samples (chlorophyll maximum) spanning the deployment period to confirm that *P. fraudulentus* was the dominant species throughout the bay. Cell abundances were determined based on species-specific standard curves.

| Sample information | | | benchtop SHA probes (cells l ⁻¹) | | | |
|--------------------|--------------------|-------|--|--|--|--|
| Location | Date | Depth | <i>P. australis</i> (auD1 ^a) | <i>P. fraudulentus</i> (frD2 ^b) | <i>P. multiseris</i> / <i>P. pseudodelicatissima</i> (muD2 ^b) | <i>P. pungens</i> (pung1 ^b) |
| ESP North | September 10, 2013 | 15 m | – | 3.29 × 10 ⁵ | – | – |
| | September 16, 2013 | 10 m | – | – | – | – |
| | September 23, 2013 | 5 m | – | 7.40 × 10 ⁴ | – | – |
| ESP South | September 10, 2013 | 10 m | – | 6.47 × 10 ⁵ | – | < 5000 |
| | September 17, 2013 | 7 m | – | 2.73 × 10 ⁵ | – | – |
| | September 23, 2013 | 7 m | – | 9.11 × 10 ⁴ | – | – |
| Monterey Wharf | September 11, 2013 | 7.5 m | – | 8.30 × 10 ⁵ | – | ~5000 |
| | September 30, 2013 | 5 m | – | 2.06 × 10 ⁴ | – | – |
| South entry to Bay | September 11, 2013 | 14 m | < 5000 | 9.15 × 10 ⁵ | – | – |
| | September 17, 2013 | 12 m | – | 3.29 × 10 ⁵ | – | – |
| | September 19, 2013 | 12 m | – | 4.41 × 10 ⁵ | – | – |

^a Scholin et al. (1999).

^b Bowers et al. (2017).

australis, *P. multiseris*/*P. pseudodelicatissima* and *P. pungens*) were negative or less than 5000 cells l⁻¹ (Table 1). Probe results for *P. australis*, *P. multiseris* and *P. multiseris*/*P. pseudodelicatissima* on both ESPs were all negative. Cell abundances of the *Pseudo-nitzschia* 'seriata' size class (which includes *P. fraudulentus*) based on microscopy counts at MW during this same time frame ranged from 1.51 × 10⁴ to 4.50 × 10⁵ cells l⁻¹ (Fig. 2c).

In stark contrast, samples acquired in 2015 via shipboard bottle casts (surface and DCM; n = 151), Dorado AUV gulper samples (2 transects; n = 29), and in situ measurements onboard two ESPs (n = 42) demonstrated that pDA within the bay ranged from 10² to 10⁴ ng l⁻¹ and was consistently higher in the southern sampling locations

(Figs. 3 and 4a). The time series provided by the two ESPs documented an average pDA concentration three times higher at ESP south, while the average chlorophyll concentration was fifty percent higher at ESP north (Table 2). Culturing efforts (n = ~300 isolates) confirmed that *P. australis* was the dominant species present. Probe results from both ESPs supported this finding and also revealed a background population of *P. fraudulentus* (Fig. 4a). The average *P. australis* cell abundance was higher at ESP south compared to ESP north (Table 2; 6.30 × 10⁵ cells l⁻¹ versus 3.90 × 10⁵ cells l⁻¹), while the average *P. fraudulentus* cell concentration was approximately the same at both ESP locations (Table 2; Fig. 4a). All other species (*P. arenysensis*, *P. multiseris*, *P. multiseris*/*P. pseudodelicatissima* and *P. pungens*) were at or below the

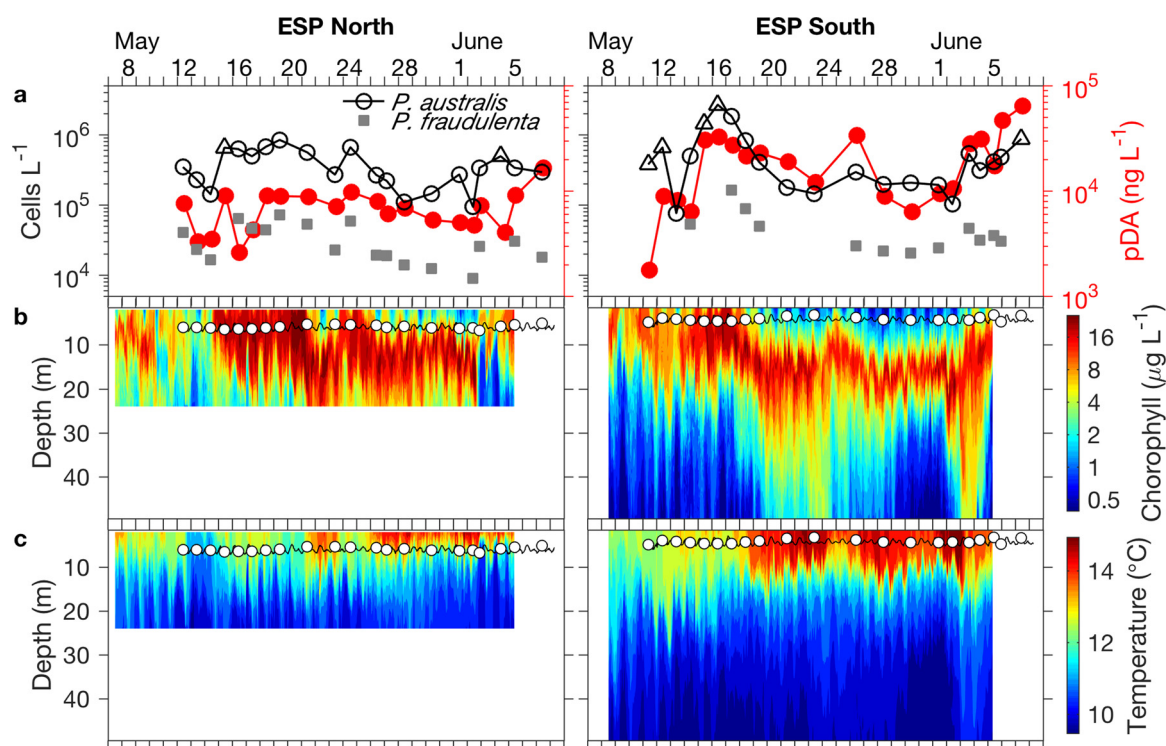


Fig. 4. Results from ESP time-series in northern and southern Monterey Bay. (a) Abundance estimates from the two detectable *Pseudo-nitzschia* probes overlaid with pDA. Time points where HAB arrays were saturated with *P. australis* cells are represented with triangles; those cell abundances are considered as minimum values. Wire walkers deployed at each ESP location captured vertical chlorophyll (b) and temperature (c) profiles. White circles in (b) and (c) indicate depth and time of ESP samples.

Table 2

Comparison of average measurements by the two ESPs deployed in 2015. ¹Averages include unknown error due to saturation of some assay results (as outlined in Fig. 4a).

| | Cell Abundance (10 ⁵ cells l ⁻¹) | | pDA (ug l ⁻¹) | chl a (ug l ⁻¹) | Water Clarity (% Trans.) | Temp. (°C) | Salinity (‰) |
|-----------|--|-----------------------|------------------------------|--------------------------------|-----------------------------|---------------|-----------------|
| | <i>P. australis</i> ¹ | <i>P. fraudulenta</i> | | | | | |
| ESP North | 3.90 | 0.48 | 7.0 | 11.5 | 74 | 13.0 | 33.6 |
| ESP South | 6.30 | 0.49 | 21.2 | 5.8 | 84 | 13.8 | 33.6 |

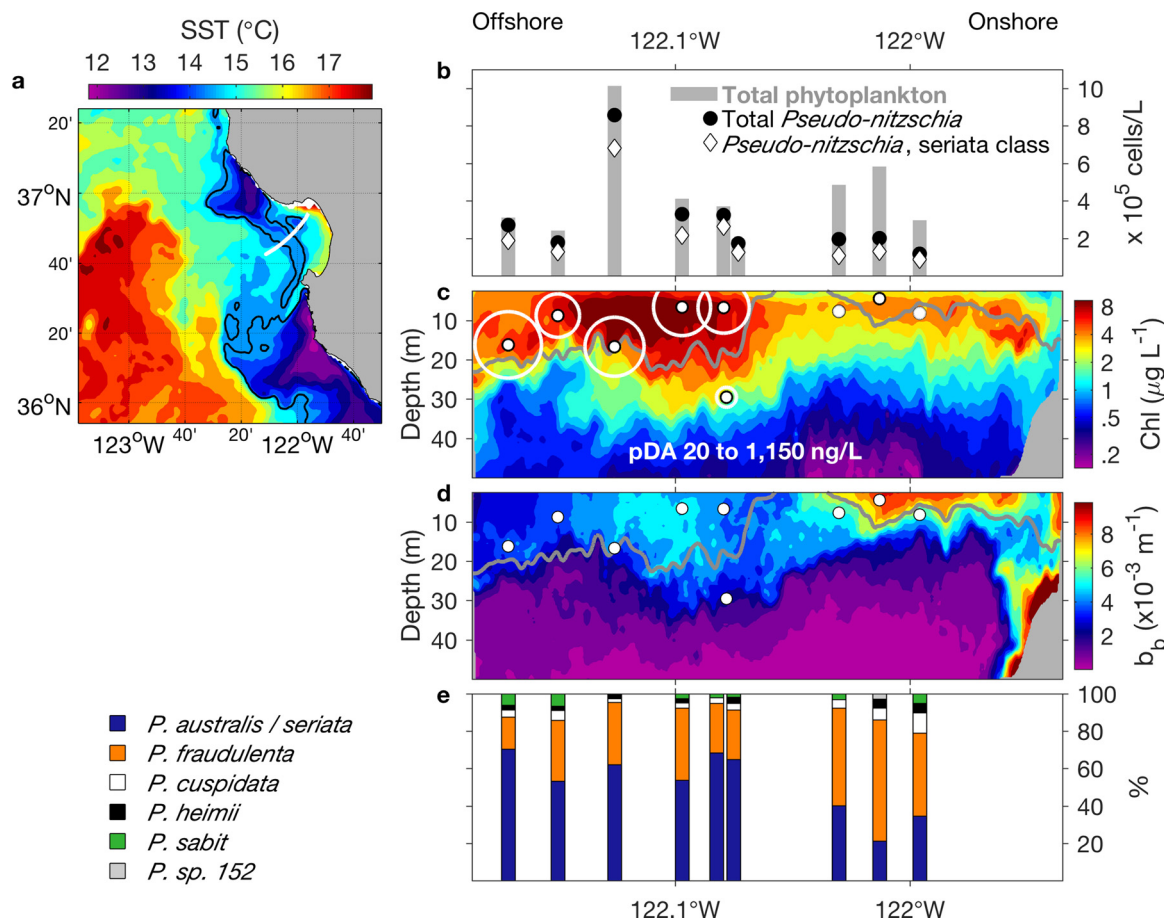


Fig. 5. (a) Dorado AUV transect crossed a cold upwelling filament on September 16, 2013 as depicted by the white line in the sea surface temperature (SST) map. The black contour line is the 14.65 °C isotherm. (b) Concentrations of total phytoplankton, total *Pseudo-nitzschia*, and ‘seriate’ size class *Pseudo-nitzschia* for each AUV sample based on light microscopy. (c) Chlorophyll distribution within the water column; the grey line is the 13.5 °C isotherm, illustrating the upwelling filament location where the isotherm outcrops to the surface; solid white circles indicate the locations of AUV water sampling; open white circles represent the concentration of particulate domoic acid (pDA) in each sample. Note: the sixth sample depicted outside the chlorophyll maximum was a targeted control sample. (d) Backscatter properties of the inshore and offshore phytoplankton populations. (e) Species composition based on DNA fingerprinting analysis (ARISA relative fluorescence).

limit of detection of the arrays (Greenfield et al., 2008; Bowers et al., 2017). Light transmission, temperature, and salinity were comparable at the two ESP locations (Table 2). Moored WireWalker profilers deployed at the two locations revealed that deployment of the ESPs occurred during a strong upwelling event, when HAB populations were most abundant in the mixed layer (Fig. 4b). With subsequent relaxation of upwelling, populations descended into a concentrated subsurface layer in the thermocline/nutricline (Fig. 4b, c). A primary distinction between the two sites was that the mixed layer remained warmer and deeper at ESP south, and sampling was generally occurring above the pronounced DCM (Fig. 4c).

3.3. AUV mapping and sampling of pDA and *Pseudo-nitzschia*

On September 16, 2013, the Dorado AUV was deployed to map

environmental conditions and phytoplankton distributions, and to autonomously target sampling within chlorophyll maximums along a section extending from outside Monterey Bay onto the northern shelf in the bay, ending at the northern ESP (Fig. 5). This survey transected a cold water filament resulting from upwelling (Fig. 5a). Onboard measurements of water column structure coupled with downstream analyses revealed two distinct populations. The offshore phytoplankton community was dominated by *Pseudo-nitzschia*, which consisted primarily of ‘seriate’ size class cells (Fig. 5b) and was marked by higher chlorophyll fluorescence and pDA (Fig. 5c). Higher optical backscatter (Fig. 5d) and lower *Pseudo-nitzschia* abundances were observed in the inshore population (Fig. 5b). ARISA results indicated a shift in dominance from *P. australis/P. seriate* (150 base pair [bp] peak) offshore to *P. fraudulenta* (203 bp) inshore (Fig. 5e), the latter result supporting observations from SHA and culturing as outlined above. Other species

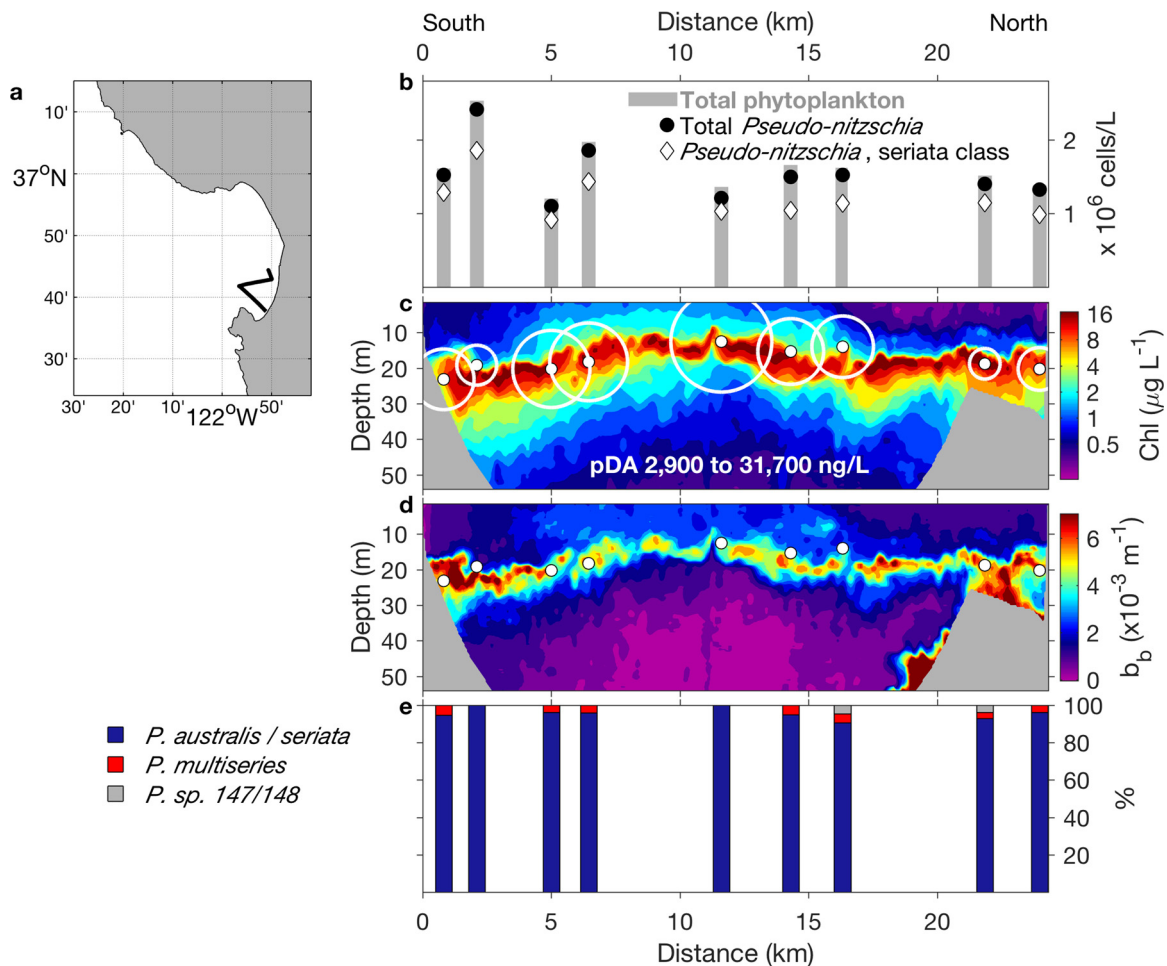


Fig. 6. Dorado AUV transect across the south bay on May 28, 2015 depicted by the black line in Fig. 6a (cloud cover precluded inclusion of SST data). (b) Concentrations of total phytoplankton, total *Pseudo-nitzschia*, and ‘seriata’ size class *Pseudo-nitzschia* for each AUV sample based on light microscopy. (c) Chlorophyll distribution within the water column depicting a deep maximum layer; solid white circles indicate the locations of AUV water sampling; open white circles represent the concentration of particulate domoic acid (pDA) in each sample. (d) Optical backscattering, further distinguishing phytoplankton populations across the upwelling filament. (e) Relative abundance of *Pseudo-nitzschia* species based on DNA fingerprinting analysis (ARISA).

detected (in both populations) were *P. cuspidata* (233 bp), *P. heimii* (195 bp), *P. sabit* (138 bp) and an unknown *Pseudo-nitzschia* sp. (152 bp).

On May 28, 2015, the Dorado AUV was deployed along a zigzag transect across the southern shelf in Monterey Bay (Fig. 6a) in response to the higher pDA and *P. australis* concentrations that were being reported in real-time by the southern ESP. The phytoplankton community was comprised predominately of *Pseudo-nitzschia*, which in turn was dominated across all samples by ‘seriata’ size class cells (Fig. 6b). High pDA persisted in a deep subsurface chlorophyll layer (Fig. 6c) and ranged over an order of magnitude independent of *Pseudo-nitzschia* abundance (2.63×10^2 – 2.10×10^3 pDA cell⁻¹). Backscatter was uniform throughout this layer (Fig. 6d), consistent with homogeneity of the populations in the layer. ARISA results demonstrated that the *Pseudo-nitzschia* community was dominated by *P. australis*/*P. seriata*, with *P. multiseriata* and an unknown putative *Pseudo-nitzschia* sp. (147–148 bp) comprising a very small part of the assemblage (Fig. 6e).

3.4. Shifts in species and toxicity during the 2015 bloom

Ninety-seven *Pseudo-nitzschia* strains were isolated from January through April 2015 prior to initiation of the bloom period (defined as April 29th when *P. australis* abundances at SCW first exceeded 5×10^4 cells l⁻¹) and were assigned to the following species based on sequencing of the LSU locus: *P. australis* (n = 19), *P. delicatissima* ‘c’

(n = 1), *P. fraudulenta* (n = 13), *P. multiseriata* (n = 34), *P. pungens* (n = 29), and *P. seriata* (n = 1). From April 29th to July 7th, two hundred twenty-seven strains were established and assigned to the following species based on sequencing of the LSU locus: *P. australis* (n = 207), *P. delicatissima* ‘a’ (n = 3), *P. fraudulenta* (n = 2), *P. multiseriata* (n = 3), and *P. seriata* (n = 12). Subsets of isolates from both time periods were used to determine pDA cell⁻¹ (Table 3). Isolates of *P. australis* consistently exhibited the highest cellular toxin content during both pre-bloom and bloom periods, followed by *P. seriata* and *P. pungens*, which were present only during the pre-bloom period.

A shift in species abundance spanning the beginning of the bloom period was captured in two data sets. First, benchtop sandwich hybridization assays on shipboard bottle cast samples (surface and DCM) from ten sites demonstrated the shift in abundance for three species: *P. multiseriata*/*P. pseudodelicatissima*, *P. australis*, and *P. fraudulenta* (Fig. 7a, b). Second, whole cell hybridization probes on weekly samples collected at the SCW site revealed an abrupt shift from *P. multiseriata* / *P. pseudodelicatissima* to *P. australis* between April 22, 2015 and April 29, 2015, with pDA values trending with *P. australis* concentrations (Fig. 7c). Although the muD2 probe detects *P. pseudodelicatissima* (Miller and Scholin, 1996; in particular *P. hasleana* within this species complex [Bowers et al., 2017]), the labeled cells in this study belonged to the larger ‘seriata’ size class and were therefore assigned as *P. multiseriata*.

Table 3

Pseudo-nitzschia species isolated in 2015, with a subset used to measure pDA per cell. Strains isolated before the bloom period started (defined as April 29, 2015 when *P. australis* abundances at Santa Cruz Wharf first exceeded 5×10^4 cells L^{-1}) are in the grey shaded boxes.

| | Isolates | | | | pg DA cell ⁻¹ | |
|------------------|-----------------------------|--------------|------------|-----------------|--------------------------|---------|
| | Species | no. isolates | no. tested | mean +/- st dev | minimum | maximum |
| PRE-BLOOM PERIOD | <i>P. australis</i> | 19 | 3 | 2.176 +/- 1.871 | 0.227 | 3.958 |
| | <i>P. delicatissima</i> 'c' | 1 | 0 | n/a | n/a | n/a |
| | <i>P. fraudulenta</i> | 13 | 1 | 0.001 | n/a | n/a |
| | <i>P. multiseriata</i> | 34 | 7 | 0.002 +/- 0.001 | 0.001 | 0.003 |
| | <i>P. pungens</i> | 29 | 8 | 0.021 +/- 0.017 | 0.004 | 0.049 |
| | <i>P. seriata</i> | 1 | 1 | 0.131 | n/a | n/a |
| BLOOM PERIOD | <i>P. australis</i> | 207 | 16 | 0.324 +/- 0.327 | 0.0301 | 1.007 |
| | <i>P. delicatissima</i> 'a' | 3 | 1 | 0.005 | n/a | n/a |
| | <i>P. fraudulenta</i> | 2 | 1 | 0.003 | n/a | n/a |
| | <i>P. multiseriata</i> | 3 | 1 | 0.002 | n/a | n/a |
| | <i>P. seriata</i> | 12 | 4 | 0.983 +/- 0.744 | 0.029 | 1.783 |

4. Discussion

Efforts to understand the ecological dynamics of *Pseudo-nitzschia* blooms have been hampered by a lack of species resolution within existing observational records. While more than thirteen species (of forty-nine described globally to date) have been documented within Monterey Bay (Bates et al., 1998; Bigelow and Leslie, 1930; Cupp, 1943; Horner et al., 1997; Lelong et al., 2012 [review]; Lundholm et al., 2006; Villac et al., 1993; Walz et al., 1994; Trainer et al., 2000; this study), detection methodologies and monitoring efforts have focused primarily on *P. australis* and *P. multiseriata*, given historical events (e.g. Bates et al., 1989; Scholin et al., 2000; McCabe et al., 2016). Intra-species variability in per cell toxin quotas for these and other documented domoic acid producers (Trainer et al., 2012; Lelong et al., 2012), ongoing descriptions of new toxic and non-toxic species (e.g. Lim et al., 2012, 2013; Lundholm et al., 2012; Harðardóttir et al., 2015; Percopa et al., 2016; Teng et al., 2014, 2016), and documented shifts in global distributions (e.g. Jester et al., 2009; Lundholm et al., 2010; Lelong et al., 2012) and bloom events (e.g. Schnetzer et al., 2007; Trainer et al., 2009; Du et al., 2016; McCabe et al., 2016) all support the need to address species diversity within the complexities of bloom initiation, persistence, and decline (Thorel et al., 2017).

A multidisciplinary approach combining traditional and emergent detection methodologies, with an array of mobile and stationary platforms capable of in situ adaptive sampling, can enhance understanding of bloom dynamics with respect to intensity, spatial scale, duration, toxicity, and species composition. To that end, this study utilized strategic deployment of platforms and high-resolution detection capabilities to uncover several interesting patterns in potential species relationships across small-scale spatial and temporal scales within Monterey Bay during two very different *Pseudo-nitzschia* bloom events. While both the 2013 and 2015 bloom periods were dominated by the larger 'seriata' size class of *Pseudo-nitzschia* cells, analyses documented blooms dominated by low DA-producing *P. fraudulenta* and highly toxic *P. australis*, respectively. With respect to variability in overall species

composition and toxin distribution between the two years, the study design revealed unique spatial (2013) and temporal (2015) patterns.

During the 2013 study period, the bay was persistently dominated by low toxin producing *P. fraudulenta*, while an offshore patch was dominated by more toxic *P. australis*. These two unique populations, revealed by AUV-targeted sampling and high-resolution genetic methods, were located within just a few kilometers of each other and were defined by differences in species diversity, cell abundance, and toxicity. A cold upwelling filament that existed as part of an offshore eddy separated the two populations. It is plausible that such offshore bloom populations are a source for delivery of cells into Monterey Bay, as described for the Pacific Northwest coast (Trainer et al., 2009). Blooms of *P. fraudulenta* have not been documented in Monterey Bay, although this species has been identified routinely in samples (e.g. Buck et al., 1992; Cangelosi et al., 1997; Miller and Scholin, 1998; Scholin et al., 1999) and was potentially part of historically described *Nitzschia* assemblages (e.g. Bolin and Abbott, 1963). Low toxicity has been reported for isolates of *P. fraudulenta* in select locations (this study, Rhodes et al., 1998; Wells et al., 2005; Thessen et al., 2009), and thus far documented blooms have not been toxic (e.g. Rines et al., 2002; Gárate-Lizárraga et al., 2007). Isolates obtained during this study were at the low end of cellular DA quotas ($< 1/1000$ of *P. australis*).

During the 2015 study period, samples from early spring exhibited diversity in *Pseudo-nitzschia* species; however, in late spring there was an abrupt shift to a bloom dominated by *P. australis*. This bloom was part of an unprecedented west coast-wide toxic event (McCabe et al., 2016) coincident with the persistent northeast Pacific warm anomaly (Bond et al., 2015; Di Lorenzo and Mantua, 2016). McCabe et al. (2016) identified the warm anomaly as a key factor in the 2015 HAB in the northern California Current System, where unusually warm water was linked to northward range expansion of *P. australis*. This species is a common inhabitant of Monterey Bay, therefore range expansion was not a local factor. Rather, high biomass was driven by a strong spring upwelling transition, followed by intermittent upwelling that periodically rejuvenated HAB populations that were retained within Monterey

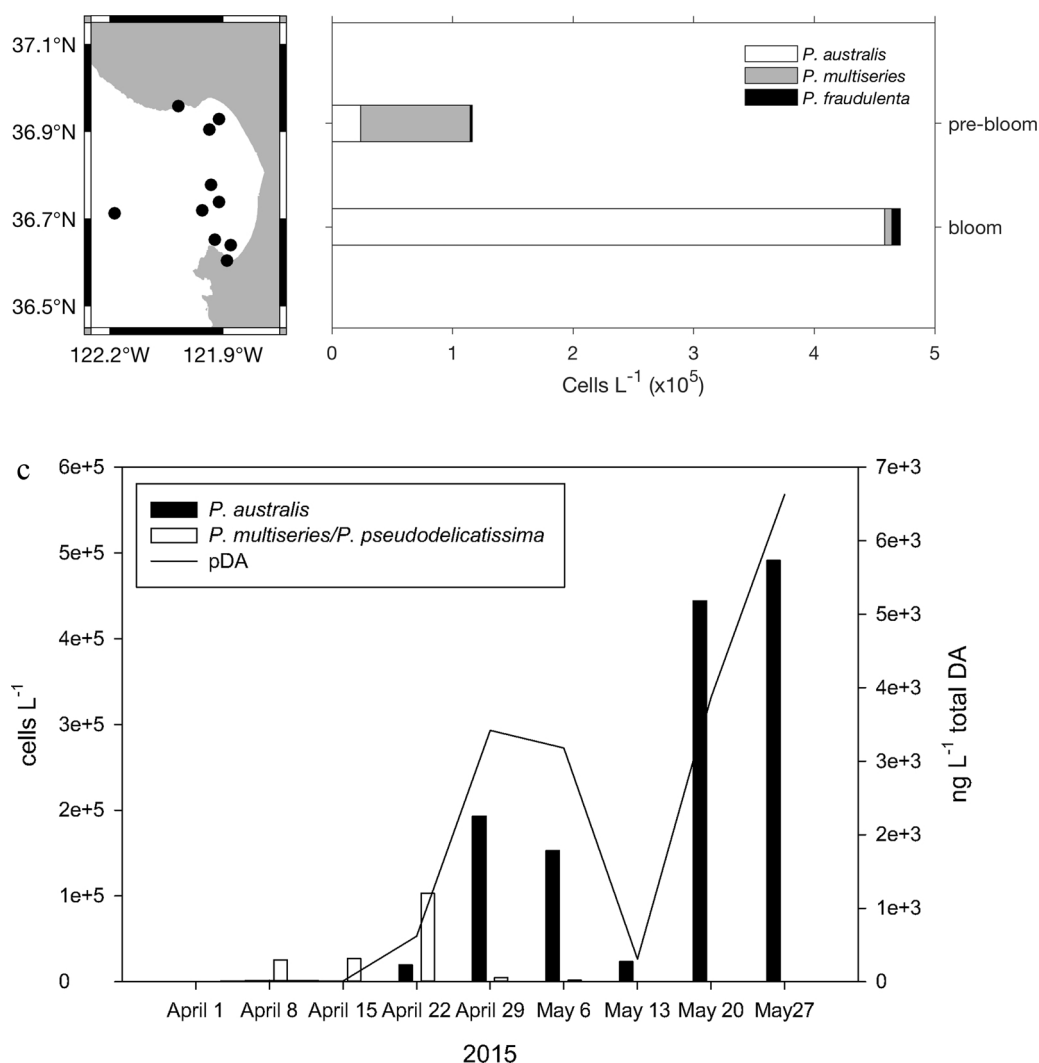


Fig. 7. Shifts in dominant *Pseudo-nitzschia* species, uncovered by benchtop sandwich hybridization assays on samples from ten stations (a,b) and whole cell hybridization at the Santa Cruz Wharf (c). Period 1 includes samples prior to the start of the bloom (April 29, 2015), while Period 2 includes samples from after the start of the event (b). Whole cell hybridization probes on weekly samples collected at the Santa Cruz Wharf captured the shift from *P. multiseriis/P. pseudodelicatissima* to *P. australis* between April 22, 2015 and April 29, 2015 (c).

Bay (Ryan et al., 2017). High toxicity was driven by anomalous background nutrient ratios, specifically exceptionally low ratios of silicate to nitrate. Interestingly, Thorel et al. (2017) documented a recent non-toxic bloom of *P. delicatissima* associated with a low ratio of silicate to nitrate in the Bay of Seine (France), despite the occurrence of *P. australis* in that region. During the 2015 Monterey Bay toxic event, silicate exhaustion, coincident with available nitrate, was observed in association with subsurface HAB layers. Accumulations of high DA concentrations linked to silicate limitation are in agreement with previous laboratory and field studies (Bates et al., 1991; Pan et al., 1996a, 1996b; Anderson et al., 2006; Schnetzer et al., 2007). It is interesting to note that the anomalous environment in Monterey Bay selected for *P. australis* and not one of the other four DA-producing species present prior to the bloom, in particular *P. multiseriis*, which had exceeded bloom threshold concentrations at SCW. In recent years *P. multiseriis* has been a rare component of the phytoplankton assemblage in Monterey Bay (K. Hayashi and G.J. Smith, unpubl. data), mirroring the overall global decline in this species (Lelong et al., 2012).

Shifts in *Pseudo-nitzschia* species composition and abundance leading into the 2015 HAB event in Monterey Bay were consistent with observations made further north off Oregon and Washington coasts. In Monterey Bay, *P. australis* transitioned from a minor to a prominent

component of the assemblage during the latter part of April (10^5 cells l^{-1} range), consistent with an increase in the number of *P. australis* isolates established prior to (20%) and during the bloom event (91%). Samples from Newport, OR showed that *P. australis* did not dominate the *Pseudo-nitzschia* community (35%) in early to mid-April (McCabe et al., 2016). Twice monthly phytoplankton counts along the Newport Hydrographic transect captured a transition in mid-May from 'medium' sized *Pseudo-nitzschia* cells to the 'wide' cell size group (which includes *P. australis*; Du et al., 2016). The first detection of DA in razor clams near Newport was on April 21st (Du et al., 2016). Coincidentally, on April 29th, DA concentrations in mussels off of Santa Cruz (CA) first exceeded the regulatory limit (McCabe et al., 2016). *P. australis* was also a relatively minor constituent of the *Pseudo-nitzschia* community (22%) in samples from Kalaloch, WA collected early to mid-April (McCabe et al., 2016). Beginning in May this species comprised over 90% of the total *Pseudo-nitzschia* assemblage (10^5 cells l^{-1} range) off Long and Kalaloch beaches (McCabe et al., 2016). Taken together, these findings suggest that regional phytoplankton dynamics were connected through larger scale processes along the entire coast. The shift to *P. australis* happened nearly simultaneously over the entire region, coinciding with the spring upwelling transition that supplied nutrients to the bloom and temporarily eliminated warm anomalies throughout coastal waters of the

entire California Current System by May 2015 (Gentemann et al., 2017).

The two ESPs deployed in 2015 provided a time-series of *Pseudo-nitzschia* cell abundances and DA levels within areas of Monterey Bay that routinely exhibit enhanced chlorophyll concentrations (as determined by long-term remote sensing data, Ryan et al., 2014) relative to the rest of the bay. Both areas serve as retention zones, with periodic nutrient supplies that rejuvenate bloom populations (Ryan et al., 2008, 2009, 2011, 2014). The southern ESP recorded consistently greater concentrations of *P. australis* (2X) and pDA (3X) compared to the northern ESP, emphasizing that this secondary and smaller region of high average chlorophyll concentrations is an important location for monitoring *Pseudo-nitzschia* bloom events. The water column profiling next to each ESP demonstrated that sampling was frequently not within the chlorophyll maximum, which motivated targeted sampling of this feature by the AUV. The high-resolution mapping and targeted samples from the Dorado AUV revealed that the chlorophyll maximum extended across the entire southern shelf, and that it was almost completely dominated by *P. australis*. Further, AUV sampling targeted the most dense bloom patches and revealed maximum cell concentrations an order of magnitude greater than maximum cell concentrations from nearby MW sampling. These densest bloom patches are more representative of potential HAB impact. Particulate DA concentrations varied an order of magnitude across a distance of several kilometers and were not simply related to *Pseudo-nitzschia* abundance. This latter observation supports the need for high-resolution mapping and sampling to understand potential drivers of toxicity.

Results from consistent weekly sampling raised additional questions related to *Pseudo-nitzschia* bloom ecology. Cell counts at the MW site revealed a higher average correlation coefficient between the two size classes in 2013 (0.87) versus 2015 (0.51). Were the species comprising the ‘delicatissima’ size class significantly different in the two blooms? Do interspecies interactions influence the trajectory of a bloom? Future work using ARISA and SEM on archived samples from both years will aim to uncover how *P. fraudulenta* (2013) and *P. australis* (2015) levels fluctuated and the similarities/differences between the associated ‘delicatissima’ populations throughout the year. Such details of species associations will help advance understanding of HAB causality and contribute to improving the accuracy of predictive models.

5. Conclusions

The ability to study HAB events (initiation, persistence, and termination) across broad temporal and spatial scales provides both understanding and motivation to advance predictive skill. The sampling strategy and tools used in this study informed understanding of *Pseudo-nitzschia* population dynamics in Monterey Bay during two very different bloom years. Fine-scale shifts in diversity and toxicity were revealed, both spatial and temporal, and these observations will allow us to build on current monitoring and modeling strategies in the region. For example, newly designed molecular probes (Bowers et al., 2017) have expanded taxon-specific detection capabilities, and findings from this study will help guide their future application. A key to future work will be the mobility and adaptive sampling capabilities of platforms such as the Dorado AUV and next generation ESP (3 G; Pargett et al., 2015; Zhang et al., 2015). As major shifts in oceanic, land-sea, and atmospheric processes that have the potential to impact frequency and intensity of HABs are documented (e.g. Moore et al., 2008), high-resolution real-time data will aid marine resource management decisions and public health protection.

Disclosure

This publication does not constitute an endorsement of any commercial product or intend to be an opinion beyond scientific or other results obtained by the National Oceanic and Atmospheric

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