

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

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26

27 **Abstract**

28 Monterey Bay, California experiences near-annual blooms of *Pseudo-nitzschia* that can
29 affect marine animal health and the economy, including impacts to tourism and
30 commercial/recreational fisheries. One species in particular, *P. australis*, has been
31 implicated in the most toxic of events, however other species within the genus can
32 contribute to widespread variability in community structure and associated toxicity across
33 years. Current monitoring methods are limited in their spatial coverage as well as their
34 ability to capture the full suite of species present, thereby hindering understanding of
35 HAB events and limiting predictive accuracy. An integrated deployment of multiple in
36 situ platforms, some with autonomous adaptive sampling capabilities, occurred during
37 two divergent bloom years in the bay, and uncovered detailed aspects of population and
38 toxicity dynamics. A bloom in 2013 was characterized by spatial differences in *Pseudo-*
39 *nitzschia* populations, with the low-toxin producer *P. fraudulenta* dominating the inshore
40 community and toxic *P. australis* dominating the offshore community. An exceptionally
41 toxic bloom in 2015 developed as a diverse *Pseudo-nitzschia* community abruptly
42 transitioned into a bloom of highly toxic *P. australis* within the time frame of a week.
43 Increases in cell density and proliferation coincided with strong upwelling of nutrients.
44 High toxicity was driven by silicate limitation of the dense bloom. This temporal shift in
45 species composition mirrored the shift observed further north in the California Current
46 System off Oregon and Washington. The broad scope of sampling and unique platform

47 capabilities employed during these studies revealed important patterns in bloom
48 formation and persistence for *Pseudo-nitzschia*. Results underscore the benefit of
49 expanded biological observing capabilities and targeted sampling methods to capture
50 more comprehensive spatial and temporal scales for studying and predicting future
51 events.

52

53 **Introduction**

54

55 Members of the diatom genus *Pseudo-nitzschia* (Peragallo), comprising several
56 species with known capacity to produce the excitatory neurotoxin domoic acid (DA;
57 reviewed in Trainer et al., 2012; Lelong et al., 2012), are generally considered to have a
58 cosmopolitan distribution (Hasle, 2002). Bloom initiation, persistence and decline are
59 associated with a variety of anthropogenic influences (e.g. eutrophication, global changes
60 in water temperatures, shifts in pH) and natural forcings (e.g. advection, upwelling,
61 stratification, grazing, parasitism; for reviews see Lelong et al., 2012; Trainer et al.,
62 2012). Given the transferability of DA throughout marine food webs (e.g. Lefebvre et al.,
63 2002; Kvitek et al., 2008; Trainer et al., 2012), toxic blooms can threaten human
64 (amnesic shellfish poisoning [ASP]; Perl et al., 1990; Todd et al., 1993; Bates et al.,
65 1989, 1998) and marine mammal and bird (domoic acid poisoning [DAP]; Work et al.,
66 1993; Scholin et al., 2000) health, and severely impact local economies through closures
67 of recreational and commercial shellfish harvesting (e.g. Gallacher et al., 2001; Bill et al.,
68 2006; Smith et al., 2006; Trainer et al., 2007; Brown, 2016). Routine monitoring and
69 management efforts are hampered by challenges associated with sampling patchy

70 phytoplankton populations and limitations in morphological species identification based
71 on light microscopy. The study described herein combined high-resolution detection
72 methodologies with a network of platforms for targeted and adaptive sampling to assess
73 *Pseudo-nitzschia* community diversity and toxin dynamics throughout two different
74 bloom scenarios in Monterey Bay (California, USA).

75 Nearly every year, Monterey Bay experiences periods of *Pseudo-nitzschia* bloom
76 activity, although with varying degrees of toxin-associated impacts. For example, during
77 2013, very high cell abundances did not coincide with high DA concentrations, while in
78 2015 elevated cell abundances were associated with high DA levels as well as a period of
79 marine animal strandings and mortalities. The 2015 event was part of an unprecedented
80 bloom of *P. australis* that stretched from Santa Barbara, California to the Aleutian
81 Islands, Alaska and coincided with a regional physical anomaly, the northeast Pacific
82 “warm blob” (McCabe et al., 2016). Localized anomalous chemical conditions leading to
83 silicate depletion contributed to exceptionally high DA production in Monterey Bay *P.*
84 *australis* populations (Ryan et al., 2017), while blooms north of California were
85 associated with persistent anomalously warm temperatures that expanded the geographic
86 range of toxic *P. australis* (McCabe et al., 2016). The California Dungeness crab fishery
87 was closed for months, and estimated losses were more than \$48 million (Brown, 2016).

88 The great inter-annual variability in *Pseudo-nitzschia* ecology in Monterey Bay
89 has been revealed by long-term weekly monitoring from wharf sampling in the northern
90 and southern parts of the bay. This effort has traditionally consisted of whole cell and
91 sandwich hybridization probes for *P. australis* and *P. multiseriata/P. pseudodelicatissima*
92 (Miller and Scholin, 1998, 2000), coupled with DA measurements, on samples from the

93 northern part of the bay (SCW) and light microscopy counts of two *Pseudo-nitzschia* size
94 classes ('seriata' sized cells; valve width $\geq 3 \mu\text{m}$ and 'delicatissima' sized cells; valve
95 width $< 3 \mu\text{m}$ [Hasle, 1965; Hasle and Syvertsen, 1997]) from the south part of the bay
96 (MW). This sampling framework has uncovered long-term seasonal patterns in *Pseudo-*
97 *nitzschia* bloom activity related to environmental factors (e.g. Lane et al., 2009);
98 however, this approach lacks sufficient taxonomic resolution to differentiate the full
99 diversity of toxic species occurring in the bay. Furthermore, the methodologies used
100 exclude species once considered non-toxic that have been shown to produce DA and
101 form blooms (e.g. Adams et al., 2000, Orsini et al., 2002, Trainer et al., 2009, Trick et al.,
102 2010), and other species described more recently (e.g. Lundholm et al., 2012, Lim et al.,
103 2013; Li et al., 2017).

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

116 Monterey Bay (e.g. Hasle et al., 1972, Buck et al., 1992, Garrison et al., 1992, Villac et
117 al., 1993). While *P. australis* has been implicated as the main DA producer in the region,
118 other toxic and non-toxic *Pseudo-nitzschia* species have been identified over the past
119 several decades (e.g. Bigelow and Leslie, 1930; Cupp, 1943; Villac et al., 1993; Walz et
120 al., 1994; Horner et al., 1997; Bates et al., 1998; Lundholm et al., 2006; Lelong et al.,
121 2012 [review]). It has remained unclear how these other species fit into *Pseudo-nitzschia*
122 assemblages within Monterey Bay. The ability to fully understand community structure
123 and succession through bloom initiation, persistence and decline is thereby severely
124 restricted, which then affects downstream modeling and management efforts. It is highly
125 plausible that forecasting models (Anderson et al., 2009, 2011; Lane et al., 2009) have
126 been impacted by the current approach, leading to discrepancies such as overestimation
127 of DA levels in 2013 (C. Anderson, R. Kudela; pers. comm.).

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

138

139 **Materials and Methods**

140

141 Sample Collection

142

143 *Wharf sampling.* As part of an ongoing long-term monitoring program, samples were
144 collected weekly on the same day from the Santa Cruz (SCW; 36° 57.48' N, 122° 1.02'
145 W) and Monterey Municipal Wharves (MW; 36° 36.22' N, 121° 53.36' W) prior to and
146 throughout the study period (Figure 1). Whole water samples from SCW were collected
147 by integration of water samples collected from 3 discrete depths (0, 1.5, and 3 m) with a
148 FieldMaster 1.75 l basic water bottle (Wildco, Yulee, Florida, USA). Integrated whole
149 water samples (5, 4, 3, 2, and 1 m) from MW were collected using a 2.2 l Van Dorn water
150 sampler (Wildco, Yulee, Florida, USA). Net tows from both locations were obtained
151 using a 20 cm diameter, 20 µm mesh net to concentrate surface waters to a depth of 5 m.
152 Leading up to the start of the 2015 study, surface samples were collected with increased
153 frequency at both wharves. All samples were maintained at ambient temperature and
154 processed within two hours of arriving at the laboratory.

155

156 *Ship sampling.* Monterey Bay was sampled during two different time periods (September
157 10th to October 7th, 2013 and May 11th to June 5th, 2015) on multiple days via the R/V
158 Rachel Carson and the R/V John Martin as part of a five-year Ecology and Oceanography
159 of Harmful Algal Blooms (ECOHAB) study. Chlorophyll fluorescence was measured
160 with CTD profilers (SeaBird Electronics, Bellevue, Washington, USA) to identify and
161 sample from within and outside chlorophyll maximums using Niskin bottles (10 l – R/V

162 Rachel Carson, 5 l – R/V John Martin) mounted on a rosette. Samples were processed
163 onboard as outlined below, with the exception of water used for SHA and ARISA, which
164 was stored protected from direct light and processed at the end of the day back in the
165 laboratory.

166

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

176

177 *ESP sampling.* Details of the Environmental Sample Processor (ESP) can be found in
178 Roman et al. (2007) and Scholin et al. (2009). The moored instruments were equipped
179 with DNA and protein arrays for near real-time detection of target organisms and
180 associated toxins. For this study, one ESP was deployed in the north part of the bay
181 (36.905°N, 121.936°W) at 7 m depth, and one was deployed in the south part of the bay
182 (36.639°N, 121.879°W) at 5 m depth, from September 10th through October 21st, 2013
183 and May 10th through June 5th, 2015 (Figure 1). Both instruments were programmed to
184 collect daily samples, unless a decrease in water temperature (a proxy for upwelling

185 conditions) was detected. Detection of upwelling conditions triggered an unscheduled
186 sampling event, either autonomously by the ESP or through operator intervention. All
187 analyses were performed in situ, as described below.

188

189 Sample Processing

190

191 *FISH*. Fluorescence In Situ Hybridization (FISH) was performed on depth-integrated
192 water samples from SCW. The detailed procedure for *P. australis* (auD1), *P. multiseriis*
193 (muD1), and *P. multiseriis/P. pseudodelicatissima* (muD2) probes is outlined in Miller
194 and Scholin (1996, 1998). Cells were viewed using a Zeiss AxioImager A1 microscope
195 fitted with a fluorescein bandpass filter set (excitation 460-500 nm; emission 510-560
196 nm) and a 120 W light source (EXFO X-Cite 120). Images were acquired with a Zeiss
197 AxioCam HRc camera.

198

199 *Phytoplankton counts*. Cell counts for *Pseudo-nitzschia* were performed on net tow
200 samples collected from MW. After transport to the laboratory, 0.1 ml of 50% w/v
201 glutaraldehyde was added to 10 ml of net tow material. A Nannoplankton Counting
202 Chamber (PhycoTech, St. Joseph, Michigan, USA) was used to count cells from a 66 μ l
203 sample aliquot on an Alexis Scientific microscope through a 10X objective. *Pseudo-*
204 *nitzschia* cells were classified into two size categories (Hasle, 1965, Hasle & Syvertsen,
205 1997): the larger ‘seriata’ sized cells (valve width > 3 μ m) and the smaller ‘delicatissima’
206 sized cells (valve width < 3 μ m). Total phytoplankton community counting, including the
207 two *Pseudo-nitzschia* size classes, was carried out on gulper samples obtained by the

208 Dorado AUV. Samples were preserved in 1% acidic Lugol's upon retrieval of the Dorado
209 after a transect mission (approximately 2-4 hours). Samples were stored in amber
210 polypropylene bottles (Thermo Scientific, Wilmington, DE, USA) at 4 °C until analysis.
211 A volume of 25 ml was concentrated down to 2.5 ml via gentle centrifugation (1700 g, 15
212 min), and a volume of 1 ml of material was counted on a Sedgewick Rafter cell as
213 described above.

214

215 *Particulate Domoic Acid*. Particulate matter from 50-250 mL of whole water was
216 concentrated onto a GF/F filter (Whatman, GE Healthcare BioSciences, Pittsburgh, PA,
217 USA). Filters were placed into cryovials and stored at -80 °C until analysis, while
218 shipboard samples were first placed in liquid nitrogen, then subsequently stored at
219 -80 °C. Filters were extracted in 3 ml of 10% methanol (prepared in ultrapure water) for
220 particulate DA analysis. All extracts were sonicated for 30 s at a level of approximately
221 10 W (RMS), 0.2 µm filtered (Millex, Millipore, Billerica, MA, USA), and cleaned
222 following a solid phase extraction procedure (Wang et al., 2007). Cleaned extracts were
223 stored at 4 °C until analysis. Domoic acid analysis was conducted on an Agilent 6130
224 LC-MS system (Agilent Technologies, Santa Clara, CA, USA) with an Agilent Zorbax
225 Rapid Resolution column. The toxin was identified by the presence of a 312 amu peak in
226 positive Scanning Ion Mode (SIM) with concentration determined by signal integration
227 of the peak area and an 8-point standard curve using a certified DA standard (NRC CRM
228 DA-f).

229

230 *ARISA*. Automated Ribosomal Intergenic Spacer Analysis, a method to determine relative
231 abundances based on a ribosomal target, was performed on samples collected from
232 Dorado gulpers and ship casts. 200-500 ml were low-vacuum (5 mmHg) filtered onto 25
233 mm diameter, 0.65 µm pore size Durapore[®] membrane filters (Millipore, Cork, Ireland).
234 Filters were transferred to 2 ml polypropylene cryovials (Nalgene Nunc International,
235 Rochester, NY, USA) with sample side facing inward, snap frozen, and archived in liquid
236 nitrogen or at -80 °C. Environmental DNA samples were prepared for *ARISA* as outlined
237 in Hubbard et al. (2014). Briefly, genomic DNA was extracted using the DNeasy Plant
238 Mini Kit (Qiagen Inc., Valencia, CA, USA) and amplified and prepared for *ARISA* using
239 the *Pseudo-nitzschia*-specific ITS1 primer set PnallF (5'-TCT TCA TTG TGA ATC
240 TGA-3') and Pnall R (5'-CTT TAG GTC ATT TGG TT-3') (Hubbard et al., 2008).
241 Purification of PCR products for *ARISA* was conducted using MultiScreen- PCR₉₆ filter
242 plates (EDM Millipore, Darmstadt, Germany), and 1 ng of product was analyzed on an
243 ABI 3730 XL using a LIZ600 size standard. Electropherogram analysis with DAX
244 software (Van Mierlo Software Consultancy, Eindhoven, Netherlands) used published
245 peak calling criteria and US West Coast species assignments for peaks based on amplicon
246 length (Hubbard et al., 2008, 2014; Smith et al., 2017).

247

248 *ESP DNA and DA arrays*. The preparation of DNA and DA arrays, and protocols
249 conducted onboard the ESP, are outlined in detail elsewhere (Doucette et al., 2009;
250 Greenfield 2006, 2008). For the 2013 deployment, probes for *P. australis* (auD1), *P.*
251 *multiseries* (muD1), and *P. multiseries/P. pseudodelicatissima* (muD2) were included on
252 DNA arrays (Scholin et al., 1999). For the 2015 deployment, additional probes were

253 added to the arrays (Bowers et al., 2017): *P. arenysensis* (ary1), *P. fraudulenta* (frD2), *P.*
254 *pungens* (pung1) and an alternative probe for *P. multiseriis* (muD3). Details for
255 preparation of standard curves for determining cell abundances are outlined in Greenfield
256 et al. (2008) and Bowers et al. (2017).

257

258 *Cultures*. Multiple ‘seriata’ and ‘delicatissima’ size *Pseudo-nitzschia* chains were isolated
259 from ship casts, gulper samples, and net tows using separate, sterile disposable pipet tips
260 under a dissecting microscope (SZH10, Olympus, Japan) at 10x magnification. Chains
261 were washed two to three times with medium (0.2 µm-filtered *f/2* medium [Guillard and
262 Ryther 1962; Guillard 1975] made with Monterey Bay water amended with 106 µM
263 NaSiO₃) and transferred into individual wells of a 12-well plate (Costar) containing
264 approximately 0.5 ml of sterilized medium. The plates were incubated at 15 °C under a
265 13:11 h light:dark photoperiod. Successfully isolated cultures were transferred to 25 ml
266 borosilicate glass culture tubes containing fresh medium. When cells reached a dense
267 mid-exponential phase, DNA was extracted from a cell pellet and the large ribosomal
268 subunit was sequenced as described by Bowers et al. (2016). In order to determine
269 cellular particulate DA (pDA), representative species in mid-exponential phase were
270 inoculated into fresh medium in triplicate in a step-wise manner to achieve a final volume
271 of 1 l. Before harvest, cultures were inspected for clumping and health of cells. Two 50
272 ml aliquots of each culture replicate were low-vacuum (5 mm Hg) filtered onto 25 mm
273 diameter, 0.65 µm pore size Durapore[®] membrane filters (Millipore). Filters were
274 transferred to 2 ml polypropylene cryovials (Nalgene Nunc International, Rochester, NY,
275 USA), snap frozen in liquid nitrogen and archived at -80 °C. An aliquot from each flask

276 was preserved with 1% acidic Lugol's in scintillation vials and stored protected from
277 light until cell counts were performed. For DA analysis, manufacturer's protocol supplied
278 with the Domoic Acid Test Kit (Mercury Science, Raleigh, NC, USA) was followed.
279 Samples were prepared by adding 1 ml of DI water to cryovials containing filters and
280 sonicating 3 x 10 sec at 30% power on ice (Heat Systems, Farmingdale, NY, USA). Cell
281 counts on replicates were performed using a 1 ml Sedgwick Rafter counting chamber
282 (Pyser SGI Ltd., Kent, UK), with a minimum of three rows and 250 total cells counted.
283
284 *Benchtop Sandwich Hybridization Assays*. Sandwich hybridization was performed on
285 samples collected from boat casts and the wharf sites. From whole water, multiple 500 ml
286 volumes were low-vacuum (5 mm Hg) filtered onto 25 mm diameter, 0.65 µm pore size
287 Durapore[®] membrane filters (Millipore). Filters were transferred to a 2 ml polypropylene
288 cryovial (Nalgene Nunc International, Rochester, NY, USA) with sample side facing
289 inward, snap frozen and archived in liquid nitrogen for downstream sandwich
290 hybridization assays. Details for preparing and running SHA plates are outlined in
291 Harvey (2014) and elsewhere (Scholin et al., 1999; Goffredi et al., 2006; Haywood et al.,
292 2007; Marin and Scholin, 2010). Preparation of standard curves for estimating cell
293 abundances is also described elsewhere (Greenfield et al., 2008, Bowers et al., 2017).
294 Sandwich hybridization plates for 2013 samples were prepared with a combination of the
295 following probes: *P. australis* (auD1), *P. fraudulenta* (frD2), *P. multiseriata*/*P.*
296 *pseudodelicatissima* (muD2) and *P. pungens* (pung1) [Scholin et al., 1999, Bowers et al.,
297 2017]. Sandwich hybridization plates for 2015 samples were prepared with a combination
298 of the following probes: *P. arenysensis* (ary1), *P. australis* (auD1), *P. fraudulenta* (frD2),

299 *P. multiseriata* (muD1), *P. multiseriata/P. pseudodelicatissima* (muD2, muD3), and *P.*
300 *pungens* (pung1) [Scholin et al., 1999, Bowers et al., 2017].

301

302 **Results**

303

304 *Shore Station Monitoring*

305

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

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

322 results from SCW (northern bay, Figure 1) exhibiting a combined average abundance of
323 3.30×10^4 cells l^{-1} for *P. australis*, *P. multiseriis* and *P. pseudodelicatissima* in 2015,
324 which was more than two orders of magnitude higher than the average of 1.90×10^2 cells
325 l^{-1} in 2013 (Figure 2c,d). Note, although combined whole cell hybridization data for all
326 species are shown in Figure 2c and 2d, values from the muD2 probe (*P. multiseriis*, *P.*
327 *pseudodelicatissima*) were negligible and contributed to 2015 data only on the following
328 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
329 cells l^{-1}], April 29 [4.85×10^3 cells l^{-1}] and May 6 [1.65×10^3 cells l^{-1}].

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

338

339 *Targeted and Adaptive Sampling – pDA and Pseudo-nitzschia species throughout the bay*

340

341 Broader spatial resolution of sampling during the approximate one-month
342 deployment windows reflected the low (2013) and high (2015) pDA values at SCW. In
343 2013, shipboard bottle casts (surface and DCM [deep chlorophyll maximum]; n=104),
344 Dorado AUV gulper samples (12 transects; n=85) and in situ measurements onboard two

345 ESPs (n=29) revealed zero to trace amounts of pDA within the bay, but identified a toxic
346 (up to 10^3 ng l⁻¹) population concentrated offshore (Figure 3a). Culturing efforts (n=~500
347 isolates) and SHA performed on a subset of bottle cast samples (n=11; temporal [seven
348 dates] and spatial [four sites]) confirmed that *P. fraudulenta* was the dominant species
349 within the bay, as it was detected in ten of eleven samples (2.06×10^4 to 9.15×10^5 cells
350 l⁻¹), while the remaining probes (*P. australis*, *P. multiseriis*/*P. pseudodelicatissima* and
351 *P. pungens*) were negative or less than 5000 cells l⁻¹ (Table 1). Probe results for *P.*
352 *australis*, *P. multiseriis* and *P. multiseriis*/*P. pseudodelicatissima* on both ESPs were all
353 negative. Cell abundances of the *Pseudo-nitzschia* 'seriata' size class (which includes *P.*
354 *fraudulenta*) based on microscopy counts at MW during this same time frame ranged
355 from 1.51×10^4 to 4.50×10^5 cells l⁻¹ (Figure 2c).

356 In stark contrast, samples acquired in 2015 via shipboard bottle casts (surface and
357 DCM; n=151), Dorado AUV gulper samples (2 transects; n=29), and in situ
358 measurements onboard two ESPs (n=42) demonstrated that pDA within the bay ranged
359 from 10^2 to 10^4 ng l⁻¹ and was consistently higher in the southern sampling locations
360 (Figures 3 and 4a). The time series provided by the two ESPs documented an average
361 pDA concentration three times higher at ESP south, while the average chlorophyll
362 concentration was fifty percent higher at ESP north (Table 2). Culturing efforts (n=~300
363 isolates) confirmed that *P. australis* was the dominant species present. Probe results from
364 both ESPs supported this finding and also revealed a background population of *P.*
365 *fraudulenta* (Figure 4a). The average *P. australis* cell abundance was higher at ESP south
366 compared to ESP north (Table 2; 6.30×10^5 cells l⁻¹ versus 3.90×10^5 cells l⁻¹), while the
367 average *P. fraudulenta* cell concentration was approximately the same at both ESP

368 locations (Table 2; Figure 4a). All other species (*P. arenysensis*, *P. multiseriis*, *P.*
369 *multiseriis*/*P. pseudodelicatissima* and *P. pungens*) were at or below the limit of
370 detection of the arrays (Greenfield et al., 2008, Bowers et al., 2017). Light transmission,
371 temperature, and salinity were comparable at the two ESP locations (Table 2). Moored
372 WireWalker profilers deployed at the two locations revealed that deployment of the ESPs
373 occurred during a strong upwelling event, when HAB populations were most abundant in
374 the mixed layer (Figure 4b). With subsequent relaxation of upwelling, populations
375 descended into a concentrated subsurface layer in the thermocline / nutricline (Figure
376 4b,c). A primary distinction between the two sites was that the mixed layer remained
377 warmer and deeper at ESP south, and sampling was generally occurring above the
378 pronounced DCM (Figure 4c).

379

380 *AUV mapping and sampling of pDA and Pseudo-nitzschia*

381

382 On September 16, 2013, the Dorado AUV was deployed to map environmental
383 conditions and phytoplankton distributions, and to autonomously target sampling within
384 chlorophyll maximums along a section extending from outside Monterey Bay onto the
385 northern shelf in the bay, ending at the northern ESP (Figure 5). This survey transected a
386 cold water filament resulting from upwelling (Figure 5a). Onboard measurements of
387 water column structure coupled with downstream analyses revealed two distinct
388 populations. The offshore phytoplankton community was dominated by *Pseudo-nitzschia*,
389 which consisted primarily of ‘seriata’ size class cells (Figure 5b) and was marked by
390 higher chlorophyll fluorescence and pDA (Figure 5c). Higher optical backscatter (Figure

391 5d) and lower *Pseudo-nitzschia* abundances were observed in the inshore population
392 (Figure 5b). ARISA results indicated a shift in dominance from *P. australis/P. seriata*
393 (150 base pair [bp] peak) offshore to *P. fraudulenta* (203 bp) inshore (Figure 5e), the
394 latter result supporting observations from SHA and culturing as outlined above. Other
395 species detected (in both populations) were *P. cuspidata* (233 bp), *P. heimii* (195 bp), *P.*
396 *sabit* (138 bp) and an unknown *Pseudo-nitzschia* sp. (152 bp).

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

409

410 *Shifts in species and toxicity during the 2015 bloom*

411

412 Ninety-seven *Pseudo-nitzschia* strains were isolated from January through April
413 2015 prior to initiation of the bloom period (defined as April 29th when *P. australis*

414 abundances at SCW first exceeded 5×10^4 cells l^{-1}) and were assigned to the following
415 species based on sequencing of the LSU locus: *P. australis* (n=19), *P. delicatissima* ‘c’
416 (n=1), *P. fraudulenta* (n=13), *P. multiseriata* (n=34), *P. pungens* (n=29), and *P. seriata*
417 (n=1). From April 29th to July 7th, two hundred twenty-seven strains were established and
418 assigned to the following species based on sequencing of the LSU locus: *P. australis*
419 (n=207), *P. delicatissima* ‘a’ (n=3), *P. fraudulenta* (n=2), *P. multiseriata* (n=3), and *P.*
420 *seriata* (n=12). Subsets of isolates from both time periods were used to determine pDA
421 cell⁻¹ (Table 3). Isolates of *P. australis* consistently exhibited the highest cellular toxin
422 content during both pre-bloom and bloom periods, followed by *P. seriata* and *P.*
423 *pungens*, which were present only during the pre-bloom period.

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

435

436 **Discussion**

437

438 Efforts to understand the ecological dynamics of *Pseudo-nitzschia* blooms have
439 been hampered by a lack of species resolution within existing observational records.
440 While more than thirteen species (of forty-nine described globally to date) have been
441 documented within Monterey Bay (Bates et al., 1998; Bigelow and Leslie, 1930; Cupp
442 1943; Horner et al., 1997; Lelong et al., 2012 [review]; Lundholm et al., 2006; Villac et
443 al., 1993; Walz et al., 1994; Trainer et al., 2000; this study), detection methodologies and
444 monitoring efforts have focused primarily on *P. australis* and *P. multiseriata*, given
445 historical events (e.g. Bates et al., 1989; Scholin et al., 2000; McCabe et al., 2016). Intra-
446 species variability in per cell toxin quotas for these and other documented domoic acid
447 producers (Trainer et al., 2012; Lelong et al., 2012), ongoing descriptions of new toxic
448 and non-toxic species (e.g. Lim et al., 2012; 2013; Lundholm et al., 2012; Harðardóttir et
449 al., 2015; Percopo et al., 2016; Teng et al., 2014; 2016), and documented shifts in global
450 distributions (e.g. Jester et al., 2009; Lundholm et al., 2010; Lelong et al., 2012) and
451 bloom events (e.g. Schnetzer et al., 2007; Trainer et al., 2009; Du et al., 2016; McCabe et
452 al., 2016) all support the need to address species diversity within the complexities of
453 bloom initiation, persistence, and decline (Thorel et al., 2017).

454 A multidisciplinary approach combining traditional and emergent detection
455 methodologies, with an array of mobile and stationary platforms capable of in situ
456 adaptive sampling, can enhance understanding of bloom dynamics with respect to
457 intensity, spatial scale, duration, toxicity, and species composition. To that end, this study
458 utilized strategic deployment of platforms and high-resolution detection capabilities to
459 uncover several interesting patterns in potential species relationships across small-scale

460 spatial and temporal scales within Monterey Bay during two very different *Pseudo-*
461 *nitzschia* bloom events. While both the 2013 and 2015 bloom periods were dominated by
462 the larger ‘seriata’ size class of *Pseudo-nitzschia* cells, analyses documented blooms
463 dominated by low DA-producing *P. fraudulenta* and highly toxic *P. australis*,
464 respectively. With respect to variability in overall species composition and toxin
465 distribution between the two years, the study design revealed unique spatial (2013) and
466 temporal (2015) patterns.

467 During the 2013 study period, the bay was persistently dominated by low toxin
468 producing *P. fraudulenta*, while an offshore patch was dominated by more toxic *P.*
469 *australis*. These two unique populations, revealed by AUV-targeted sampling and high-
470 resolution genetic methods, were located within just a few kilometers of each other and
471 were defined by differences in species diversity, cell abundance, and toxicity. A cold
472 upwelling filament that existed as part of an offshore eddy separated the two populations.
473 It is plausible that such offshore bloom populations are a source for delivery of cells into
474 Monterey Bay, as described for the Pacific Northwest coast (Trainer et al., 2009). Blooms
475 of *P. fraudulenta* have not been documented in Monterey Bay, although this species has
476 been identified routinely in samples (e.g. Buck et al., 1992, Cangelosi et al., 1997, Miller
477 and Scholin, 1998; Scholin et al., 1999) and was potentially part of historically described
478 *Nitzschia* assemblages (e.g. Bolin and Abbott, 1963). Low toxicity has been reported for
479 isolates of *P. fraudulenta* in select locations (this study, Rhodes et al., 1998, Wells et al.,
480 2005, Thessen et al., 2009), and thus far documented blooms have not been toxic (e.g.
481 Rines et al., 2002, Gárate-Lizárraga et al., 2007). Isolates obtained during this study were
482 at the low end of cellular DA quotas (< 1/1000 of *P. australis*).

483 During the 2015 study period, samples from early spring exhibited diversity in
484 *Pseudo-nitzschia* species; however, in late spring there was an abrupt shift to a bloom
485 dominated by *P. australis*. This bloom was part of an unprecedented west coast-wide
486 toxic event (McCabe et al., 2016) coincident with the persistent northeast Pacific warm
487 anomaly (Bond et al., 2015; Di Lorenzo and Mantua, 2016). McCabe et al. (2016)
488 identified the warm anomaly as a key factor in the 2015 HAB in the northern California
489 Current System, where unusually warm water was linked to northward range expansion
490 of *P. australis*. This species is a common inhabitant of Monterey Bay, therefore range
491 expansion was not a local factor. Rather, high biomass was driven by a strong spring
492 upwelling transition, followed by intermittent upwelling that periodically rejuvenated
493 HAB populations that were retained within Monterey Bay (Ryan et al., 2017). High
494 toxicity was driven by anomalous background nutrient ratios, specifically exceptionally
495 low ratios of silicate to nitrate. Interestingly, Thorel et al. (2017) documented a recent
496 non-toxic bloom of *P. delicatissima* associated with a low ratio of silicate to nitrate in the
497 Bay of Seine (France), despite the occurrence of *P. australis* in that region. During the
498 2015 Monterey Bay toxic event, silicate exhaustion, coincident with available nitrate, was
499 observed in association with subsurface HAB layers. Accumulations of high DA
500 concentrations linked to silicate limitation are in agreement with previous laboratory and
501 field studies (Bates et al., 1991; Pan et al., 1996a; 1996b; Anderson et al., 2006;
502 Schnetzer et al., 2007). It is interesting to note that the anomalous environment in
503 Monterey Bay selected for *P. australis* and not one of the other four DA-producing
504 species present prior to the bloom, in particular *P. multiseriis*, which had exceeded
505 bloom threshold concentrations at SCW. In recent years *P. multiseriis* has been a rare

506 component of the phytoplankton assemblage in Monterey Bay (K. Hayashi and G.J.
507 Smith, unpubl. data), mirroring the overall global decline in this species (Lelong et al.,
508 2012).

509 Shifts in *Pseudo-nitzschia* species composition and abundance leading into the
510 2015 HAB event in Monterey Bay were consistent with observations made further north
511 off Oregon and Washington coasts. In Monterey Bay, *P. australis* transitioned from a
512 minor to a prominent component of the assemblage during the latter part of April (10^5
513 cells l^{-1} range), consistent with an increase in the number of *P. australis* isolates
514 established prior to (20%) and during the bloom event (91%). Samples from Newport,
515 OR showed that *P. australis* did not dominate the *Pseudo-nitzschia* community (35%) in
516 early to mid-April (McCabe et al., 2016). Twice monthly phytoplankton counts along the
517 Newport Hydrographic transect captured a transition in mid-May from ‘medium’ sized
518 *Pseudo-nitzschia* cells to the ‘wide’ cell size group (which includes *P. australis*; Du et
519 al., 2016). The first detection of DA in razor clams near Newport was on April 21st (Du et
520 al., 2016). Coincidentally, on April 29th, DA concentrations in mussels off of Santa Cruz
521 (CA) first exceeded the regulatory limit (McCabe et al., 2016). *P. australis* was also a
522 relatively minor constituent of the *Pseudo-nitzschia* community (22%) in samples from
523 Kalaloch, WA collected early to mid-April (McCabe et al., 2016). Beginning in May this
524 species comprised over 90% of the total *Pseudo-nitzschia* assemblage (10^5 cells l^{-1} range)
525 off Long and Kalaloch beaches (McCabe et al., 2016). Taken together, these findings
526 suggest that regional phytoplankton dynamics were connected through larger scale
527 processes along the entire coast. The shift to *P. australis* happened nearly simultaneously
528 over the entire region, coinciding with the spring upwelling transition that supplied

529 nutrients to the bloom and temporarily eliminated warm anomalies throughout coastal
530 waters of the entire California Current System by May 2015 (Gentemann et al., 2017).

531 The two ESPs deployed in 2015 provided a time-series of *Pseudo-nitzschia* cell
532 abundances and DA levels within areas of Monterey Bay that routinely exhibit enhanced
533 chlorophyll concentrations (as determined by long-term remote sensing data, Ryan et al.,
534 2014) relative to the rest of the bay. Both areas serve as retention zones, with periodic
535 nutrient supplies that rejuvenate bloom populations (Ryan et al., 2008; 2009; 2011;
536 2014). The southern ESP recorded consistently greater concentrations of *P. australis*
537 (2X) and pDA (3X) compared to the northern ESP, emphasizing that this secondary and
538 smaller region of high average chlorophyll concentrations is an important location for
539 monitoring *Pseudo-nitzschia* bloom events. The water column profiling next to each ESP
540 demonstrated that sampling was frequently not within the chlorophyll maximum, which
541 motivated targeted sampling of this feature by the AUV. The high-resolution mapping
542 and targeted samples from the Dorado AUV revealed that the chlorophyll maximum
543 extended across the entire southern shelf, and that it was almost completely dominated by
544 *P. australis*. Further, AUV sampling targeted the most dense bloom patches and revealed
545 maximum cell concentrations an order of magnitude greater than maximum cell
546 concentrations from nearby MW sampling. These densest bloom patches are more
547 representative of potential HAB impact. Particulate DA concentrations varied an order of
548 magnitude across a distance of several kilometers and were not simply related to *Pseudo-*
549 *nitzschia* abundance. This latter observation supports the need for high-resolution
550 mapping and sampling to understand potential drivers of toxicity.

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

561

562 **Conclusions**

563

564 The ability to study HAB events (initiation, persistence, and termination) across
565 broad temporal and spatial scales provides both understanding and motivation to advance
566 predictive skill. The sampling strategy and tools used in this study informed
567 understanding of *Pseudo-nitzschia* population dynamics in Monterey Bay during two
568 very different bloom years. Fine-scale shifts in diversity and toxicity were revealed, both
569 spatial and temporal, and these observations will allow us to build on current monitoring
570 and modeling strategies in the region. For example, newly designed molecular probes
571 (Bowers et al., 2017) have expanded taxon-specific detection capabilities, and findings
572 from this study will help guide their future application. A key to future work will be the
573 mobility and adaptive sampling capabilities of platforms such as the Dorado AUV and

574 next generation ESP (3G; Pargett et al., 2015; Zhang et al., 2015). As major shifts in
575 oceanic, land-sea, and atmospheric processes that have the potential to impact frequency
576 and intensity of HABs are documented (e.g. Moore et al., 2008), high-resolution real-
577 time data will aid marine resource management decisions and public health protection.

578

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580

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586

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588 This publication does not constitute an endorsement of any commercial product or intend
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Sample information			benchttop SHA probes (cells l ⁻¹)			
Location	Date	Depth	<i>P. australis</i> (auD1 ¹)	<i>P. fraudulenta</i> (frD2 ²)	<i>P. multiseri</i> / <i>P. pseudodelicatissima</i> (muD2 ¹)	<i>P. pungens</i> (pung1 ²)
ESP North	September 10, 2013	15 m	--	3.29 x 10 ⁵	--	--
	September 16, 2013	10 m	--	--	--	--
	September 23, 2013	5 m	--	7.40 x 10 ⁴	--	--
ESP South	September 10, 2013	10 m	--	6.47 x 10 ⁵	--	<5000
	September 17, 2013	7 m	--	2.73 x 10 ⁵	--	--
	September 23, 2013	7 m	--	9.11 x 10 ⁴	--	--
Monterey Wharf	September 11, 2013	7.5 m	--	8.30 x 10 ⁵	--	~5000
	September 30, 2013	5 m	--	2.06 x 10 ⁴	--	--
South entry to Bay	September 11, 2013	14 m	<5000	9.15 x 10 ⁵	--	--
	September 17, 2013	12 m	--	3.29 x 10 ⁵	--	--
	September 19, 2013	12 m	--	4.41 x 10 ⁵	--	--

¹Scholin et al., 1999.

²Bowers et al., 2017.

Table 1. Benchttop SHA was performed on several ship cast samples (chlorophyll maximum) spanning the deployment period to confirm that *P. fraudulenta* was the dominant species throughout the bay. Cell abundances were determined based on species-specific standard curves.

	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

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 Figure 4a).

	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 'c'</i>	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 'a'</i>	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

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.

