Harmful Algae 92 (2020) 101617

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Is San Francisco Bay resistant to *Pseudo-nitzschia* and domoic acid?

Raphael M. Kudela*, Kendra Hayashi, Cristian Garrido Caceres

Ocean Sciences Department, 1156 High Street, University of California, Santa Cruz, CA 95064, United States

ARTICLE INFO

Keywords:
Pseudo-nitzschia australis
Pseudo-nitzchia multiseries
San Francisco Bay
Domoic acid
Temperature
Salinity
Trace metals

ABSTRACT

San Francisco Bay (SFB), California, USA is the largest estuary in the western United States and is home to more than 7 million people in nine counties and 101 cities. It is highly nutrient enriched and is directly connected to the Gulf of the Farallones and coastal Pacific ocean through the Golden Gate strait. The Gulf of the Farallones is one of several "hotspots" for the neurotoxin domoic acid, produced by members of the genus *Pseudo-nitzschia*. Despite the close proximity, SFB has few reports of harmful algal blooms and low concentrations of domoic acid, suggesting that SFB is somehow resistant to toxic blooms. Here we evaluate the potential growth and toxicity of the dominant toxigenic species in California coastal waters, *P. australis* and *P. multiseries*, to directly test the hypothesis that SFB waters confer resistance to blooms. We specifically evaluate the effect of varying temperature, salinity, and to a lesser extent, nutrients on growth and toxin production. Results show equivalent growth in SFB water (maximum growth rates of 0.71 and 1.35 d^{-1} for *P. multiseries* and *P. australis*) compared to open-coast water, and comparable or greater toxicity (0 to > 100 pg DA $cell^{-1}$). The historical resistance to blooms in SFB is hypothesized to be caused by a combination of insufficient acclimation time for advected *Pseudo-nitzschia* populations to become established and suppression of toxin production in warm waters.

1. Introduction

The toxigenic diatom genus *Pseudo-nitzschia* (Peragallo) is recognized as a serious harmful algal bloom (HAB) threat globally (reviewed by Trainer et al., 2012; Lelong et al., 2012; Bates et al., 2018). This is especially true along the west coast of North America where a massive bloom in 2015 resulted in devastating impacts to ecosystem health, fisheries, and tourism (McCabe et al., 2016). The 2015 event specifically and toxic blooms generally over the past 2 decades have been linked to anomalous warm periods within the California Current System (CCS; McCabe et al., 2016; McKibben et al., 2017). There is also some evidence that the frequency and intensity of these blooms have been increasing in California (Lewitus et al., 2012) leading to heightened concern and increased awareness of coastal ecosystem health.

San Francisco Bay (SFB) is the largest estuary in California, with six sub-embayments ranging from the low salinity (0–15), river-dominated North Bay to the lagoonal South Bay where salinities range from 5-35. Within SFB, *Pseudo-nitzschia* is commonly observed at concentrations comparable to open coastal waters (Nejad et al., 2017, 2018) and SFB is recognized as being dominated by enhanced anthropogenic nutrients (Sutula et al., 2017). Past studies have linked toxic *Pseudo-nitzschia* events in California to anthropogenic nutrients (reviewed by Kudela et al., 2008, 2010; Lewitus et al., 2012) with good evidence for promotion of blooms in more enclosed embayments and coastal waters (Trainer et al., 1998, 2007). Indeed, Howard et al. (2007) identified outflow from SFB rich in urea as the likely source of enhanced toxicity for *Pseudo-nitzschia* in the adjacent Gulf of the Farallones. A recent statistical analysis of a ~30 year time series in SFB also provides evidence for increasing susceptibility of SFB to HABs driven in part by increasing anthropogenic nutrients (Sutula et al., 2017). Dissolved inorganic nitrogen (DIN) and phosphate concentrations in SFB are equal to or exceed concentrations in other estuaries, such as Chesapeake Bay, where nutrient over-enrichment has led to degraded water and aquatic habitat (Cloern and Jassby, 2012) and where domoic acid is considered a serious threat (Thessen and Stoecker, 2008; Anderson et al., 2010). Despite the persistence of *Pseudo-nitzschia* blooms throughout the CCS (Trainer et al., 2012; McCabe et al., 2016; Smith et al., 2018), within SFB there are very few reports of elevated domoic acid toxicity. Domoic acid is ubiquitous but low in SFB, and even during the 2015 west coast event, concentrations in water and mussels within SFB remained orders of magnitude lower than the open coast (Peacock et al., 2018).

The apparent resistance of SFB to domoic acid (and other HABs; Sutula et al., 2017) leads to several questions, including whether *Pseudo-nitzschia* actively grows in SFB or is merely advected in and out with estuarine circulation, and whether cell growth and toxin production is inhibited by some combination of environmental factors unique

* Corresponding author.
E-mail address: kudela@ucsc.edu (R.M. Kudela).

https://doi.org/10.1016/j.hal.2019.05.010
Received 13 February 2019; Received in revised form 22 May 2019; Accepted 23 May 2019
Available online 26 November 2019
1568-9883/

to this ecosystem. More fundamentally, the question arises whether SFB is naturally resistant to domoic acid events and whether that resistance will continue into the future. To address these questions, we evaluated the growth and toxicity as both cell quotas and toxin production rates for *P. australis* and *P. multiseries* isolated from Monterey Bay (California). Both species are frequently identified in SFB (Nejad et al., 2017, 2018) and are generally considered to be the drivers of HAB events in California (Bowers et al., 2018; Smith et al., 2018).

Some consistent patterns relevant to estuarine systems emerge when evaluating the role of environmental conditions on toxin production in *Pseudo-nitzschia*. Nutrient stress and trace metal availability consistently increase DA production, while increasing temperature and salinity are less consistent but also generally promote DA production (c.f. Lelong et al., 2012; Trainer et al., 2012; Bates et al., 2018). More recent research has highlighted the interactive effects of these parameters, particularly nitrogen, temperature and pCO_2 (Bates et al., 2018; Tatters et al., 2018). Both field and laboratory experiments have also consistently identified the stationary phase of growth as maximizing DA yield, but numerous studies have indicated that DA production also occurs during exponential growth (e.g. Kudela et al., 2004; Auro and Cochlan, 2013; McCabe et al., 2016; Radan and Cochlan, 2018).

Given this background, we chose to conduct laboratory manipulations of temperature, salinity, and to a lesser extent nutrients using both SFB water and coastal (Monterey Bay) water as a base media to determine whether physical factors (temperature, salinity) or chemical composition of the base water could be a factor in limiting growth and toxin production within SFB.

2. Materials and methods

2.1. Culture conditions

Two *Pseudo-nitzschia* species, *P. australis* MLML-5 and *P. multiseries* MW15190C3, were isolated by H. Bowers and A. Woods from Santa Cruz Municipal Wharf (16-May-2017) and Monterey Wharf (9-July-2015) respectively. The unialgal, clonal strains were maintained in 33 salinity using seawater obtained from Moss Landing Marine Laboratories (MLML) aquaculture facility 15 °C (± 0.5 °C), 120 µmol photons m^{-2} s^{-1} (determined with a Biospherical Instruments QSL-100 Quantum Scalar Irradiance meter) with 12:12 light:dark cycle using "cool white" fluorescent lamps in an environmental chamber. Stock cultures were maintained in 50 ml disposable styrene culture flasks (Fisher #430168) using MLML seawater at salinity 33.0 amended with f/2 + silicate (described below) and had been acclimated to these conditions for over 20 weeks. Experiments were conducted after transfer and acclimation (approximately 3 weeks) to triplicate borosilicate glass culture tubes for each treatment; experiments were initiated with either the acclimated culture from one of three replicates at the end of the previous experiment (salinity, temperature manipulations) or from the stock cultures. The approximate initial inoculum was 28,500 cells mL^{-1}. All culturing materials used either sterile disposables (pipettes, culture flasks) or were acid-cleaned and autoclaved prior to use. All transfers were conducted in a sterile transfer hood, but cultures were not axenic. For each experimental treatment samples were collected at varying time points with the terminal sampling occurring at least two days after stationary growth was achieved (as determined by fluorescence). Unless otherwise stated, toxicity and biomass are reported for the terminal sampling event.

Table 2
Summary of experimental conditions used to assess base water and nutrients, temperature, and salinity for *P. multiseries* MW15190C3 and *P. australis* MLML-5. Experimental conditions not listed, such as irradiance, were held constant as described in the main text. Salinity (S) is noted for individual entries where relevant, while base water is referenced as MLML, SFB18, and SFB33. Except where noted, f/2 + silicate was added to the base water after pasteurization.

	P. multiseries MW15190C3	*P. australis* MLML-5
Nutrient Amendments		
Unpasteurized water[a]	0, 25, 50, 75, 100% SFB18	–
+NH4, +EDTA		MLML (S = 24)
+Urea, +EDTA		MLML (S = 24)
+EDTA		MLML (S = 24)
+EDTA		SFB18
+EDTA		SFB33
Temperature Experiments		
10	MLML (S = 33)	MLML (S = 33)
12.5	MLML (S = 33)	MLML (S = 33)
15	MLML (S = 33)	MLML (S = 33)
18	MLML (S = 33)	–
21	MLML (S = 33)	MLML (S = 33)
24	MLML (S = 33)	MLML (S = 33)
27	MLML (S = 33)	MLML (S = 33)
Salinity Experiments		
33	MLML	MLML
30.75	MLML	–
28.75	MLML	–
27	MLML	MLML
26.25	MLML	–
24.5	SFB33	SFB33
24	SFB18	SFB18
24	MLML	MLML
22.5	MLML	MLML
18	MLML	MLML

[a] Experiment C in Fig. 1.

Media varied by experiment (Tables 1 and 2). The base cultures were maintained using seawater obtained from the MLML aquaculture facility, while water from SFB was obtained from USGS Station 18 (Central Bay; 38° 50.8′N; 121° 25.3′W, referred to as SFB18) and USGS Station 33 (South Bay; 38° 30.5′N, 121° 7.3′W, referred to as SFB33). Water was gravity-filtered (0.2-µm, Whatman PolyCap 150) and maintained at 4 °C in the dark. Prior to use, the base water was pasteurized by heating in a 20 l polypropylene carboy to 85 °C for 4 h except for one treatment where the effects of pasteurization were assessed. The pasteurized water was amended with f/2 + silicate using the Bigelow National Center for Marine Algae and Microbiota f/2 media kit (NCMA MKF250 L). For a subset of experiments the media was further amended with EDTA, urea, or ammonium using culture-grade chemicals. EDTA was added at twice the f/2 media concentration (total EDTA = 12.38 mg L^{-1}), while urea and ammonium were added to approximate SFB water, 1 µM urea (equivalent to 2 µM-N) and 10 µM ammonium. Nutrient-amended media were kept at 4 °C in the dark prior to use and was allowed to equilibrate to ambient temperatures prior to culture transfer. The pH and alkalinity of the media prior to addition of nutrients were determined with an Orion Star A300 pH meter and Hach alkalinity kit (24443-01) respectively.

Table 1
Chemical properties of base water (prior to nutrient addition).

	NO_3^- (µM)	PO_4^- (µM)	$Si(OH)_4^-$ (µM)	Urea (µM)	NH_4^+ (µM)	pH	Alkalinity	Salinity
MLML	17.49	0.95	14.87	0.10	0.77	7.78	160	33.0
SFB18	16.54	1.74	89.71	0.79	8.88	7.40	120	24.0
SFB33	50.07	4.71	88.60	1.07	8.93	7.72	140	24.5

2.2. Temperature, salinity, base water scenarios

Temperature growth experiments were conducted in the MLML base water with f/2 + silicate. Temperature was varied by adjusting three separate environmental chambers to the appropriate temperature, maintained within ± 0.5 °C, and monitored at 10 min intervals with a digital logger. Final temperature values were calculated based on the average temperature for each treatment. Light duration and intensity were adjusted to comparable values for the three environmental chambers. *P. multiseries* MW15190C3 was grown at 10, 12.5, 15, 18, 21, 24, and 27 °C while *P. australis* MLML-5 omitted the 18 °C treatment. *P. multiseries* did not exhibit positive growth above 18 °C, while *P. australis* did not exhibit positive growth above 24 °C.

Growth rates and toxicity for *P. multiseries* MW15190C3 and *P. australis* MLML-5 were assessed at multiple salinity levels including varying percentages of SFB18 or SFB33, up to 100%. Salinity was determined with a YSI EC300 salinity meter (YSI Incorporated, OH). Salinity treatments were conducted by addition of 18.2 MΩ cm (Milli-Q; EMD Millipore) water to the MLML base water (salinity 33) to produce media with salinity of 27, 24, 22.5, and 18. *P. multiseries* MW15190C3 was grown at 0, 25, 50, 75, and 100% SFB18 water mixed with MLML water in three separate experiments (labeled A, B, C in Fig. 1), with experiment C utilizing unpasteurized water. The initial salinity for SFB18 and SFB33 was 24 and 24.5 respectively resulting in additional salinity treatments of 30.75, 28.75, and 26.25 for *P. australis* MLML-5 from the SFB18 dilution treatments where SFB18 water was mixed with MLML water. *P. australis* MLML-5 was grown at 0 and 100% SFB18 and 100% SFB33, repeated a second time with 100% SFB18 and SFB33, and with urea or ammonium added to the MLML base water diluted to salinity = 24 with Milli-Q, plus addition of EDTA to all treatments (Fig. 2). For *P. multiseries* MW15190C3 only 0% and 100% SFB33 treatments were conducted. Attempts to grow both species at 16.5 were unsuccessful. Thus salinity treatments ranged from 18 to 33, with *P. australis* MLML-5 tested at 18, 22.5, 24–24.5, 26.25, 27, 28.75, 30.75, and 33, while *P. multiseries* MW15190C3 omitted the full SFB18 dilution series (salinities of 26.25, 28.75, 30.75,). The full matrix of experimental conditions is presented in Table 2.

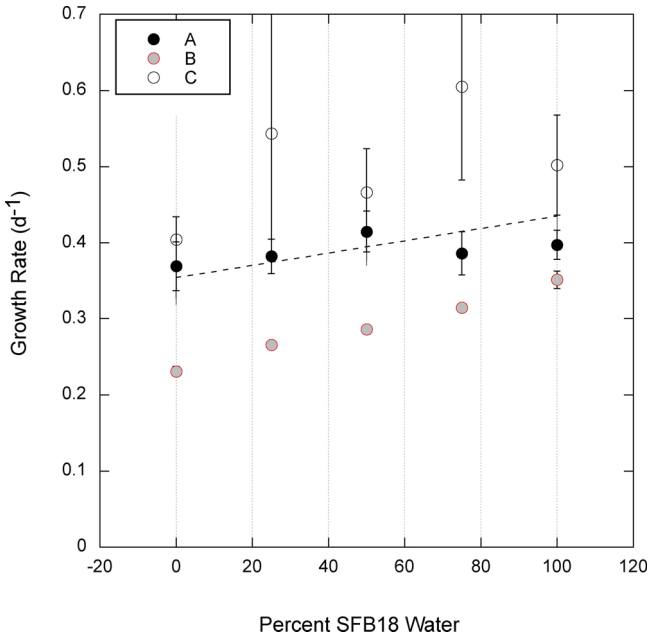

Fig. 1. Growth rates for three experiments (labeled A, B, C) using increasing percentages of SFB18 water and *P. multiseries* MW15190C3. Error bars represent standard deviation of replicate or triplicate cultures; the dashed line is a linear regression using all experimental points. The third experiment (labeled C) used unpasteurized water.

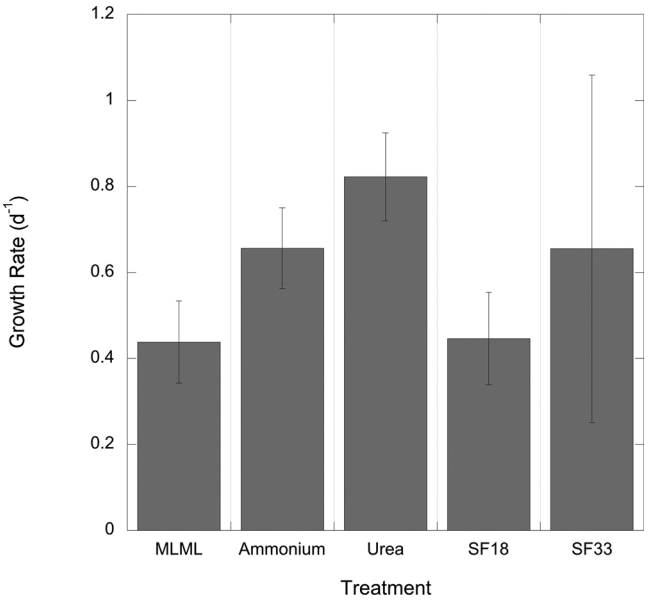

Fig. 2. Growth rates for *P. australis* MLML-5 from triplicate cultures in MLML water (MLML) amended with ammonium or ammonium and urea, or with SFB18 or SFB33 basewater. Error bars represent one standard deviation.

Experiments focused on short-term acclimation because our primary interest was in whether *Pseudo-nitzschia* advected into SFB would tolerate estuarine conditions or whether growth was even feasible; growth rates and toxicity were therefore assessed after the second transfer at a given temperature or salinity and cultures were typically not maintained beyond two transfers under a given set of conditions. At the end of the experiment the remaining culture was used to initiate the next higher temperature or lower salinity treatment. A minimum of two attempts was made to transfer cells at the tolerance limits for the cultures into the highest temperature and lowest salinity where positive growth was maintained for two transfers.

2.3. Growth rates

In vivo fluorescence of each replicate culture was measured daily using a 10AU fluorometer (Turner Designs, CA), with the culture tube placed directly into the fluorometer after 30 min dark adaptation. Cells were resuspended with gentle swirling prior to measurement. Discrete samples for cell counts were collected at various time points (typically mid-exponential growth) and at the end of each experiment. Samples (1–3 mL) were fixed with 10% final concentration Lugol's acidified iodine solution and stored at ambient temperature in the dark until being counted using a Sedgewick-Rafter chamber and a Zeiss Axiovert 200 microscope. Specific growth rates (d^{-1}), lag time (d), and maximal biomass were calculated using a modified Gompertz model with the fluorescence data (Tjørve and Tjørve, 2017), while cell counts were used to calculate toxin per cell. Q_{10} of growth rates was calculated as: $Q_{10} = (\mu_2/\mu_1)_2^{(10/(T-T)}_1$ where μ_1, μ_2 are specific growth rates (d^{-1}) at temperatures (°C) T_1, T_2 (Chaui-Berlinck et al., 2002).

2.4. Domoic acid analysis

Samples for analysis of domoic acid (DA) were collected at varying time points, with volumes adjusted based on cell density. Total domoic acid (tDA) was assessed by collecting 3–10 mL of culture material, acidifying with formic acid (final concentration 0.25%), and probe sonicating at 10 W for 30 s using a Fisher Brand D100 sonic dismembrator. Dissolved domoic acid (dDA) was determined similarly, but the culture was first syringe filtered through a 13 mm 0.2 µm PTFE filter (VWR 28145-491). For both tDA and dDA the sample was cleaned using

BondElut C18 SPE columns (Agilent 12113027) following Wang et al. (2007) and analyzed by LC–MS with Select Ion Monitoring (SIM) on an Agilent 6130 system using domoic acid certified reference material (NRC Canada) as an external standard. Particulate domoic acid (pDA) was calculated as the difference between tDA and dDA for matched samples from individual replicate cultures. Cell quotas were determined by dividing DA concentrations by cell counts from microscopy. DA production rates (R_{tox}, pg DA cell^{-1} d^{-1}) were determined as described in Zhu et al. (2017): $R_{tox} = e^{\mu} \cdot c$, where μ and c are growth rate (d^{-1}) and DA concentration per cell (pg DA cell^{-1}) respectively, with μ derived from the full experimental time-series and c obtained from the final (stationary) cell quotas. Normalized DA values were calculated separately for dDA, pDA, and tDA by dividing the appropriate DA value by the cell density, with the assumption (for dDA and tDA) that there was no loss from the media due to degradation (e.g. bacterial or photodegradation, Bouillon et al., 2006; Hagström et al., 2007); therefore the dDA and tDA quotas are based on the accumulated DA normalized to the cell numbers at a given time point.

2.5. Nutrients

Samples for nutrient analysis were collected from the various base waters (MLML, SFB18, SFB33) after gravity filtration and stored in preconditioned and cleaned 25 ml polyethylene vials at -20 °C until analysis for nitrate + nitrite (hereafter referred to as nitrate, NO_3^-), silicic acid ($Si(OH)_4^-$), and phosphate (PO_4^-) using a Lachat Instruments Flow Injection Analysis system (8000 series; Hach Co.) according to the Quick-Chem1 colorimetric techniques (Smith and Bogren, 2001; Knepel and Bogren, 2001; Wolters, 2002). Samples for ammonium (NH_4^+) and urea were collected and stored (-20 °C) in 50 ml disposable centrifuge tubes (Corning #430829) and analyzed using the fluorometric method (Holmes et al., 1999) with a TD-700 (Turner Designs, Sunnyvale, CA) fluorometer for ammonium, and a Cary 50 spectrometer (Agilent, CA) equipped with a 10 cm quartz cell following the method of Price and Harrison (1987) with modification for a longer (72 h) and lower (21 °C) digestion temperature (Mulvenna and Savidge, 1992) for urea.

2.6. Ancillary field data

For comparison with natural assemblages of *Pseudo-nitzschia*, data were analyzed from the United States Geological Survey cruises between 2014–2016 from San Francisco Bay, from ˜weekly sampling at the Santa Cruz Municipal Wharf (36° 57.48′ N122° 1.02′ W) from 2011 to 2018, and from the grow-out experiment described in McCabe et al. (2016). Field data were obtained from Nejad et al. (2018); Cloern (2019), and Schraga et al. (2018) for San Francisco Bay and Southern California Coastal Ocean Observing System (SCCOOS 2018) for Santa Cruz Municipal Wharf. Unpublished experimental data were available from the study published in McCabe et al. (2016).

2.7. Statistical analysis

Data processing and statistical analysis used a combination of Microsoft Excel (Microsoft Corp.), Kaleidagraph (Synergy Software), and MYSTAT (Systat Software). Results were considered significant when the p-value was less than $\alpha = 0.05$ for all tests.

3. Results

3.1. Effect of base media

While there was an apparent positive trend of increasing growth rate from 0 to 100% SFB18 water for *P. multiseries* MW15190C3 (Fig. 1), it was not statistically significant for either the combined data (linear regression; $p = 0.07$) or for the effect of dilution (ANOVA with repeated measures; ($p = 0.15$). There was also no significant difference in maximum biomass or lag phase ($p > 0.05$; ANOVA with repeated measures). Based on those results, *P. australis* MLML-5 was subsequently grown at 0 and 100% SFB33, and with urea or ammonium added to the MLML basewater diluted to salinity = 24, plus addition of EDTA to all treatments (Fig. 2). There was no significant difference in growth rate (ANOVA, $p > 0.05$) between 100% SFB18 and 100% SFB33 base waters for *P. australis* MLML-5 and *P. multiseries* MW15190C3 (Fig. 2) but cultures amended with urea grew significantly faster (ANOVA, $p < 0.05$) than the unamended MLML and SFB18

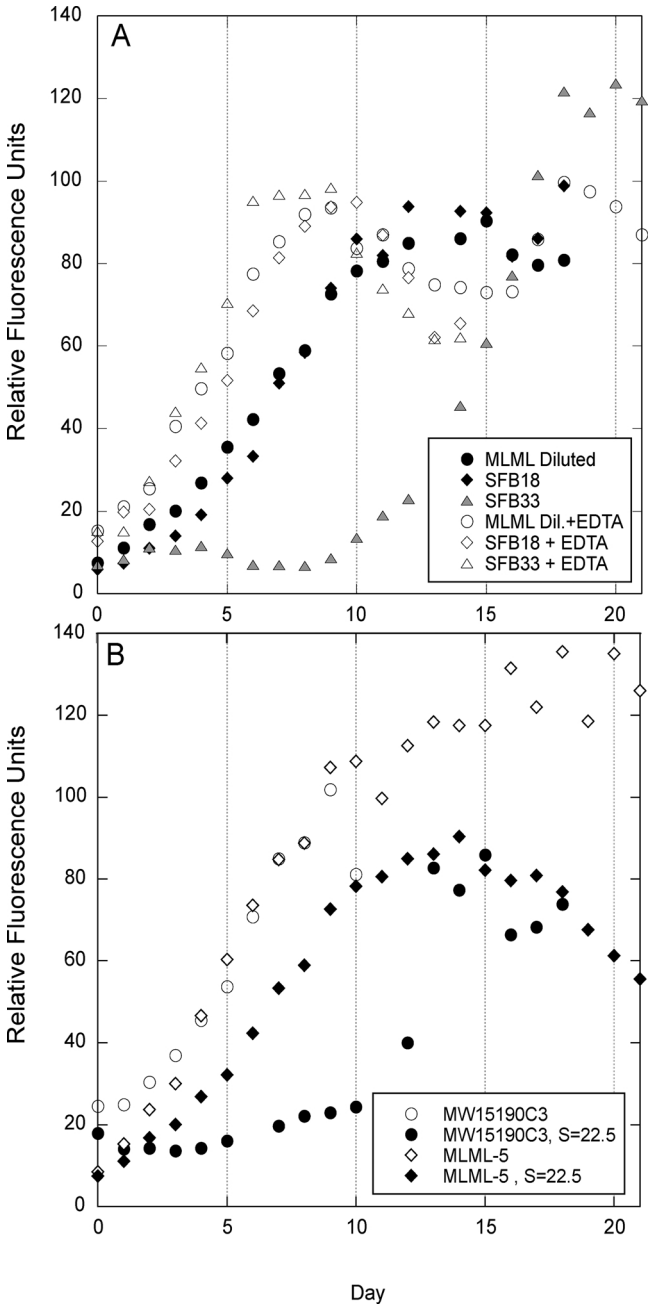

Fig. 3. Growth rates (plotted as biomass versus time) for *P. australis* MLML-5 with MLML base water diluted with Milli-Q, SFB18, and SFB33, without and with additional EDTA (A), and growth rates for *P. multiseries* MW15190C3 and *P. australis* MLML-5 at salinity of 33 and 22.5 using MLML base water with Milli-Q (B). Symbols represent the mean daily values from replicate (3x) cultures; error bars have been omitted for ease of visual interpretation of the patterns.

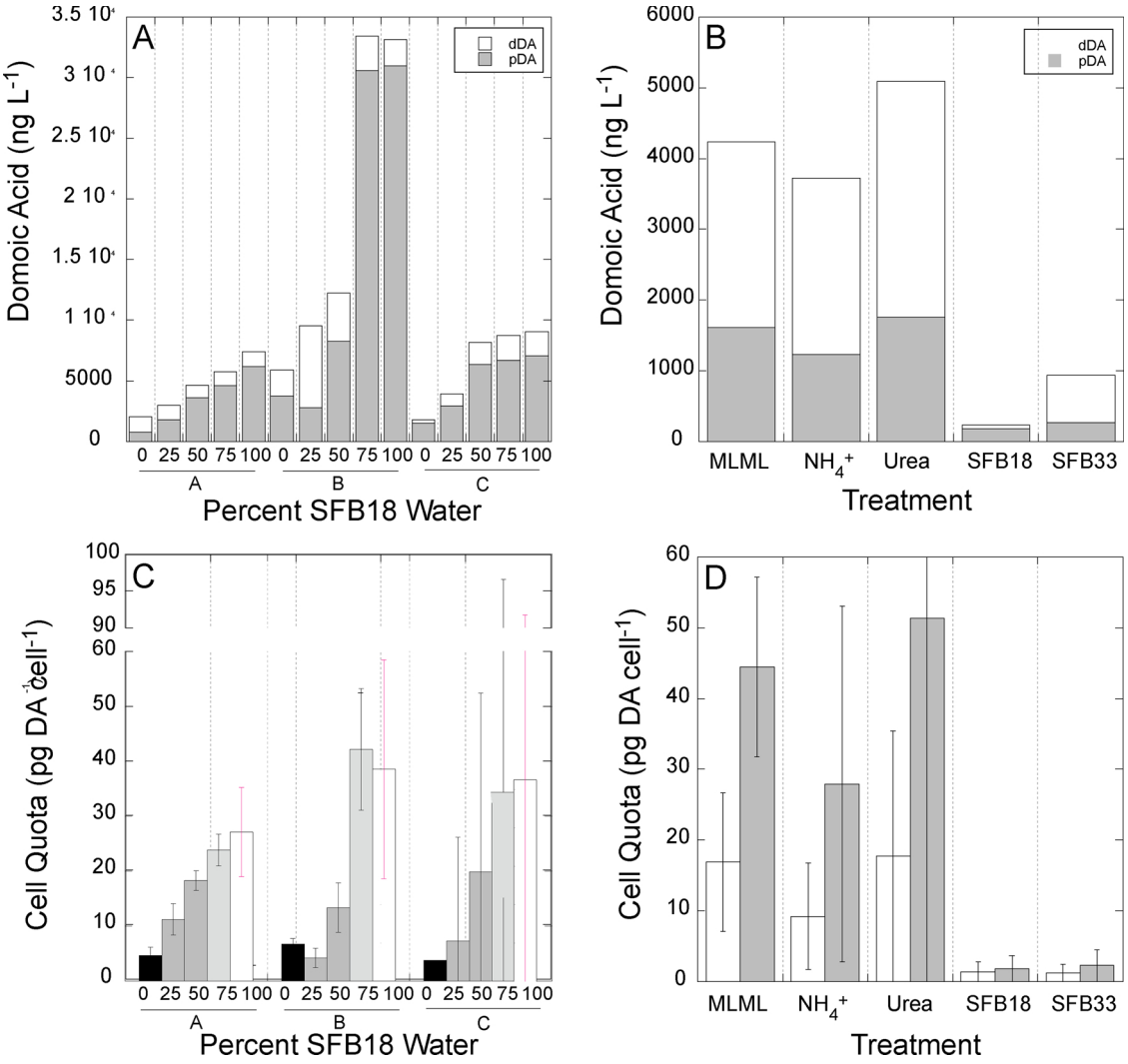

Fig. 4. Domoic acid concentrations (ng L^{-1}) for *P. multiseries* MW15190C3 (A) and *P. australis* MLML-5 (B) for particulate (shaded) and dissolved (open) domoic acid, and corresponding cell quotas (pg DA $cell^{-1}$) for *P. multiseries* MW15190C3 (C) and *P. australis* MLML-5 (D). For panel C, color of the bar denotes particulate DA quotas for individual experiments (total DA is not shown); for panel D, open and shaded bars are particulate and total DA quotas respectively. For all plots error bars represent one standard deviation of replicate cultures.

water (note that SFB18 had less urea than SFB33 prior to amendment; Table 1). There was no significant difference in maximum biomass ($p > 0.05$; ANOVA with repeated measures) across treatments. With the addition of EDTA the lag phase in SFB33 media was reduced from 12 to 0 days (Fig. 3A), while addition of EDTA had no effect on the lag phase for MLML or SFB18 base media, and growth rates and maximum biomass were not statistically different ($p > 0.05$). Lowering salinity to 22.5 also increased the lag phase to ~12 days for *P. multiseries* MW15190C3 (Fig. 3B).

Domoic acid concentration varied considerably with base media in both *P. multiseries* MW15190C3 and *P. australis* MLML-5 (Fig. 4) but with markedly different responses. For *P. multiseries* MW15190C3, increasing the concentration of SFB18 water increased toxicity but the increase was not statistically significant ($p = 0.11$; ANOVA with repeated measures). When normalized to cell number there was a strong effect of dilution for tDA and pDA ($p < 0.001$) for *P. multiseries* MW15190C3. For *P. australis* MLML-5, there was no difference between SFB18 and SFB33 but MLML media with or without added urea and ammonium was significantly higher for tDA and pDA ($p < 0.01$; ANOVA). When normalized to cell number, the differences were still significant for pDA ($p = 0.015$) and tDA ($p = 0.019$).

3.2. Effect of salinity

Growth rates and toxicity for *P. multiseries* MW15190C3 and *P. australis* MLML-5 were assessed at multiple salinity levels. As described for the dilution experiments, there was no significant effect of salinity (to a salinity of 24) on growth, biomass, or lag phase for either species. Adding the two lowest salinity levels increased the lag phase from an average of 3.5 days for *P. multiseries* MW15190C3 and 1.2 days for *P. australis* MLML-5 at salinities 24–33 to 11.5 and 12.1 days respectively at salinity 18.

As reported in *§3.1*, there was no significant difference in tDA or pDA concentration as a function of dilution with SFB18 base media for *P. australis* MLML-5, while *P. multiseries* MW15190C3 exhibited reduced toxin production. When the data were reanalyzed across all treatments that utilized salinity manipulations, including dilution of MLML media to salinity = 24, there was no significant difference for *P. australis* MLML-5 in tDA or pDA concentration or cell quota as a function of dilution (salinity), although there was a trend of increasing tDA per cell with decreasing salinity (Fig. 5). In contrast, *P. multiseries* MW15190C3 exhibited a statistically insignificant trend for increasing tDA with increasing salinity (linear regression using data from Fig. 4; $p = 0.11$) and highly significant effect of salinity for tDA (Fig. 5) and pDA normalized to cell number ($p < 0.001$; ANOVA with repeated measures).

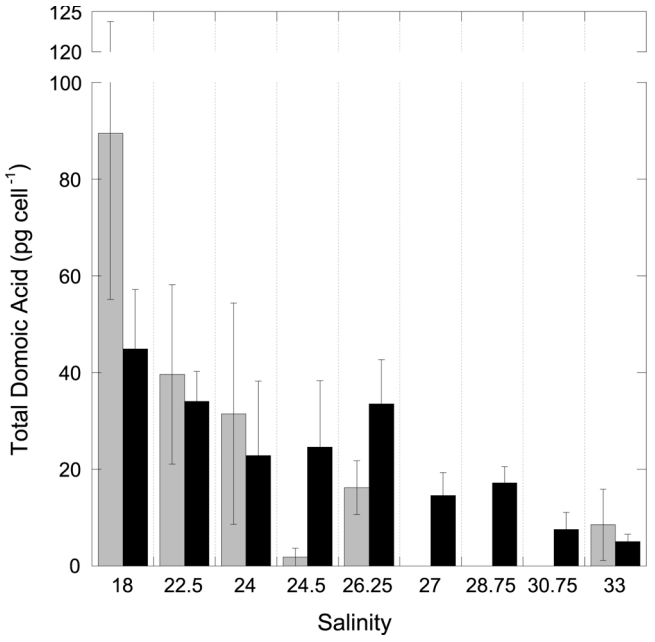

Fig. 5. Total domoic acid cell quotas (tDA; pg cell^{-1}) for *P. australis* MLML-5 (grey bars) and *P. multiseries* MW15190C3 (dark bars) across tested salinity range. Error bars represent one standard deviation of replicate cultures. Salinity was adjusted by dilution of MLML or SFB water by combining the base media and/or Milli-Q water (c.f. Table 2).

3.3. Effect of temperature

P. australis MLML-5 and *P. multiseries* MW15190C3 growth rates increased from minima of 0.27 ± 0.09 and 0.10 ± 0.01 d^{-1} at 10° respectively to a maximum of 1.35 ± 0.25 at 27° for *P. australis* MLML-5, and 0.71 ± 1.11 d^{-1} at 20° for *P. multiseries* MW15190C3 (Fig. 6). This represents Q_{10} values of 2.73 and 7.97 respectively. Narrowing the range to the more linear part of the temperature response curve for *P.*

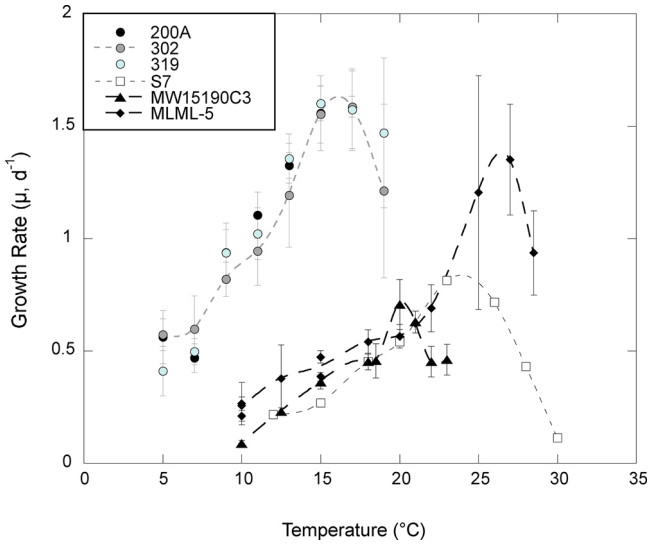

Fig. 6. Growth rate as a function of temperature for *P. australis* MLML-5 (solid diamonds) and *P. multiseries* MW15190C3 (solid triangles) compared to data reported in McCabe et al., 2016 (circles) and Zhu et al., 2017 (open squares). The dashed lines are cubic spline fits for each dataset (the McCabe et al., 2016 spline fit is for the three strains 200A, 312, and 319), while the error bars represent one standard deviation for the replicate cultures (omitted from Zhu et al., 2017). Growth rates for *P. australis* MLML-5 and *P. multiseries* MW15190C3 used MLML base water.

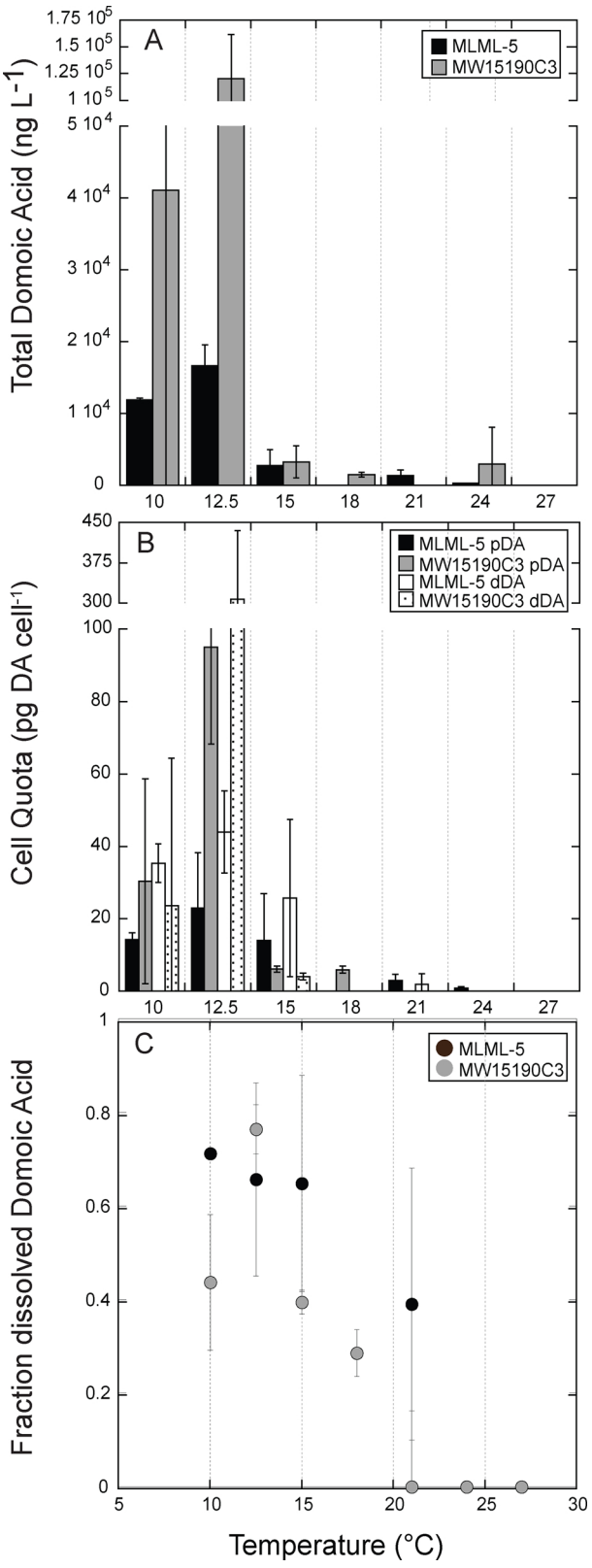

Fig. 7. Temperature effect on total domoic acid for *P australis* MLML-5 (A; black) and *P. multiseries* MW15190C3 (A; grey); cellular DA quotas for particulate (B; black) and dissolved domoic acid (B; grey) in *P. australis* MLML-5, and for particulate (B; white) and dissolved (B; stippled) DA in *P. multiseries* MW15190C3; fraction of total DA that was in the dissolved phase for *P. australis* MLML-5 (C; black) and *P. multiseries* MW15190C3 (C; grey). Error bars represent one standard deviation of replicate cultures.

multiseries MW15190C3 (12-18°) reduces the Q_{10} to what is likely a more reasonable value of 3.09. Above the optimal temperature, both strains exhibited a rapid decrease in growth rate and could not successfully be maintained at 25° for *P. multiseries* MW15190C3 and 30° for *P. australis* MLML-5. There was no significant effect of temperature on lag phase ($p > 0.05$) for either strain when using all data, but at 10 °C both strains increased the lag phase to > 10 days compared to an average of 2.5 days for all other temperatures.

Total, dissolved, and particulate DA concentrations generally decreased with increasing temperature with no detectable toxin above 24 °C, and no detectable dDA above 21 °C (Fig. 7A). Cell quotas followed the same pattern (Fig. 7B), with significant decreases in cell quota for pDA and tDA in *P. australis* MLML-5 ($p < 0.01$) and the same (but not significant) trends for *P. multiseries* MW15190C3. The ratio of dissolved to total toxin was also strongly influenced by temperature (Fig. 7C), with a significant ($p < 0.05$) trend of decreasing fraction of dDA (percent of tDA) with increasing temperature for both strains.

Data from SFB were also evaluated to examine the realized niche in temperature-salinity space (i.e. under what conditions *Pseudo-nitzschia* are present). The realized niche ranged from 23.64 to 32.90 salinity, and 10.98–23.00 °C for surface (2 m) samples (Fig. 8; Nejad et al., 2018; Cloern and Schraga, 2016; Schraga et al., 2018). Production of pDA generally increased with decreasing salinity and decreasing temperature (Table 3) but was variable. The effect of urea and NH_4^+ was only directly tested on *P. australis* MLML-5 but was indirectly manipulated in *P. multiseries* MW15190C3 since the SFB18 water used for dilution had more NH_4^+ and urea than the MLML base water. Production values were overlapping (not significantly different) when comparing MLML water versus amended media (at S = 24) for *P. australis*. For *P. multiseries* multiple linear regression identified only salinity as a significant factor, with ammonium concentrations as a weak ($p = 0.094$) but not significant factor.

3.4. Domoic acid cell quotas and production

P. australis MLML-5 and *P. multiseries* MW15190C3 exhibited a large range for toxin per cell, with pDA cell quotas ranging from 0 to > 100 pg DA cell^{-1} and tDA quotas exceeding 200 pg DA cell^{-1}. To assess whether this range is representative of natural populations, data were compared to cell quotas from Santa Cruz Municipal Wharf for 2010–2018 (Fig. 9). Cell quotas from these experiments were well within the range of variability observed at this site (mean = 41.4, median = 17.1 pg cell^{-1}, n = 205). During the 2015 event, reported cell quotas at mid-exponential growth ranged from ~7 to 21 pg cell^{-1} (McCabe et al., 2016), but maximum quotas (stationary phase) ranged from 107 to 302 pg cell^{-1} (unpublished data), also consistent with

Table 3
Particulate domoic acid production rates (pg DA cell^{-1} d^{-1}) at stationary phase as a function of treatment. Standard deviations are provided in parentheses. Bold salinity values were based on dilution of SFB18 water with MLML water, and therefore include manipulation of salinity and nutrients.

	P. multiseries MW15190C3 (SD)	*P. australis* MLML-5 (SD)
Base Water		
MLML	5.66 (0.85)	26.16 (10.81)
SFB18	39.66 (28.22)	2.14 (1.52)
SFB33	–	2.33 (1.82)
MLML + NH_4^+ (S = 24)	–	17.76 (8.27)
MLML + Urea (S = 24)	–	40.31 (19.63)
Salinity		
33	5.86 (1.53)	10.00 (67.61)
30.75	8.86 (3.58)	–
28.75	19.75 (3.44)	–
27	16.80 (4.74)	–
26.25	38.64 (9.41)	36.02 (23.82)
24[a]	39.66 (28.22)	35.75 (24.22)
22.5	40.36 (6.30)	47.39 (23.22)
18	55.56 (13.09)	106.96 (42.92)
Temperature (°C)		
10	33.23 (28.72)	18.26 (1.96)
12.5	156.95 (44.50)	33.53 (9.93)
15	8.88 (0.89)	9.49 (13.33)
18	9.39 (1.04)	–
21	0.00 (0.00)	5.74 (1.83)
24	0.00 (0.00)	2.92 (0.59)
27	0.00 (0.00)	0.00 (0.00)

[a] Salinity = 24 includes both MLML base water diluted with Milli-Q and SFB18 water.

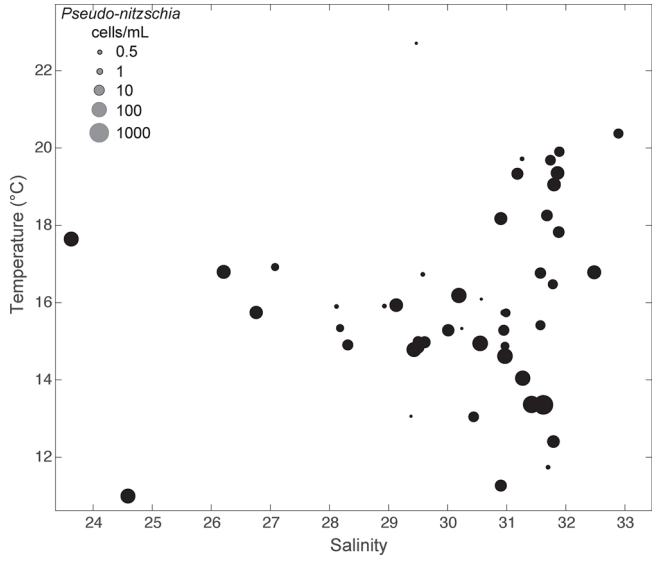

Fig. 8. Observed cell density (cells mL^{-1}) for San Francisco Bay from the USGS time series, 2014–2016, plotted in temperature-salinity space. Size of the symbols is proportional to cell density observed from microscopy counts for surface (< 5 m) water depth.

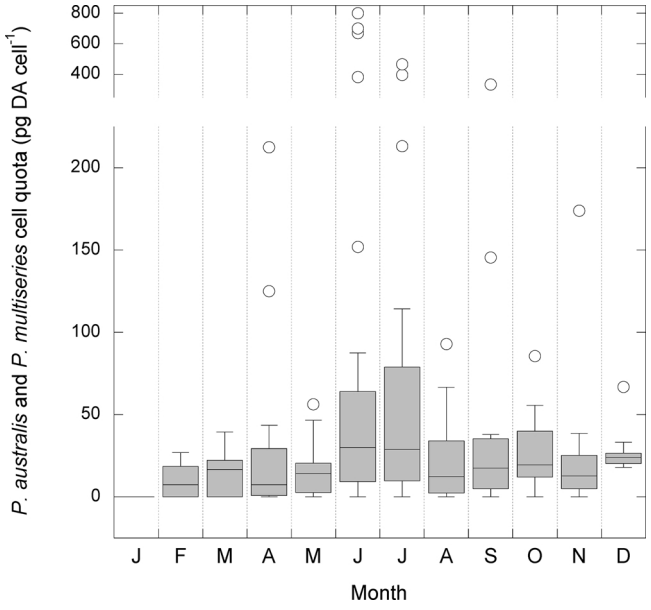

Fig. 9. Cell quotas (pg DA cell^{-1}) for toxigenic *Pseudo-nitzschia* from 2011 to 2018 at the Santa Cruz Municipal Wharf by month. Bars represent 25–75% quartiles with the solid horizontal lines indicating the median and the bars representing the 5 and 95% range, and the open circles representing statistical outliers.

these laboratory results. Finally, cell quotas from 2015 for SFB ranged from 0 to 15.5 pg cell^{-1}.

4. Discussion

SFB is physically connected to the open California coast and therefore to a source of *Pseudo-nitzschia*. The Central Bay is essentially marine water, with rapid mixing with the Gulf of the Farallones, resulting in salinity and temperature comparable to the Gulf of the Farallones. Nutrients are not typically considered limiting in SFB; indeed a trend towards increasing sensitivity to nutrient over-enrichment (Sutula et al., 2017) has been hypothesized. Irradiance is certainly a significant factor in controlling phytoplankton growth and community structure in SFB (Alpine and Cloern, 1988; Cloern, 1996; Cloern et al., 2017). While irradiance was not manipulated in this study, total irradiance of 5.84 mol photons m^{-2} d^{-1} for cultures represents ~10-30% of surface irradiance for representative sites in SFB (Alpine and Cloern, 1988; Cloern, 1996), and SFB is generally dominated by diatoms (Cloern and Dufford, 2005). For *P. multiseries* and *P. australis* the few studies that have directly examined the effect of irradiance identified a value of 100 µmol photons m^{-2} s^{-1} or higher as sufficient for growth and DA production (Bates, 1998; Cochlan et al., 2008). Toxicity generally increases with increasing irradiance (c.f. Lelong et al., 2012) and the majority of SFB would remain above the 100 µmol photons m^{-2} s^{-1} threshold under typical conditions. *P. multiseries* is also capable of surviving for weeks with no light (Mengelt and Prézelin, 2002), suggesting that irradiance is not the primary factor limiting *Pseudo-nitzschia* growth in SFB but may be influencing DA production at high levels, which may in turn promote dark survival. Thus the question remains as to why SFB is resistant to toxic blooms of *Pseudo-nitzschia* (Peacock et al., 2018), even during periods such as 2015 when there was a large source of toxigenic cells with direct connection to SFB (McCabe et al., 2016). Therefore, the focus of these experiments was to determine if some property of SFB water inhibits *Pseudo-nitzschia* growth or toxin production and whether this condition (or conditions) are likely to be maintained in the near future.

4.1. Salinity

Based on the culture experiments, *Pseudo-nitzschia* isolated from coastal California could grow in SFB water across a reasonably broad range of salinities (18–33). This laboratory range is consistent with field data where observations of cell presence identified *Pseudo-nitzschia* within a narrower realized niche (Fig. 9; ~23–33 salinity). Thessen et al. (2005) reported a similar range of 15–40 and 25–30 salinity for *P. multiseries* MU1 and MU7, also isolated from Monterey Bay. Our results are entirely consistent with the assertion that *Pseudo-nitzschia* is euryhaline and halotolerant (Lelong et al., 2012) but with considerable species and strain variability (Thessen et al., 2005; Lelong et al., 2012; Pednekar et al., 2018).

Very few studies have examined the effect of salinity on DA production. Doucette et al. (2008) examined growth and toxicity for *P. multiseries* MU7 at 10, 20, 30, and 40 salinity, but in contrast to this study, allowed the cultures to become fully acclimated with at least 6 generations for each treatment. Cell quotas for DA (tDA, dDA, pDA) and DA production rates were highest at 30–40 salinity in that study. The authors proposed that DA production declined at lower salinity values in response to energy partitioning towards osmotic balance. Pednekar et al. (2018) conducted experiments with *P. pungens* SP-1 isolated from western India, and reported similar trends of increasing tDA and production with increasing salinity, corresponding to maximal growth rates at 15–30 salinity. Ayache et al. (2019) used abrupt salinity shocks with *P. australis* IFR-PAU-16.1 and IFR-PAU-16.2. They reported that there was considerable interspecific variability, with DA both increasing and decreasing with salinity, and considerable release of dDA in part due to abrupt osmotic shock lysing cells.

In contrast to these previous findings our data show an increase in DA with decreasing salinity with the highest cell quotas and production at the lowest salinity tested (Table 3). However, the patterns were not statistically consistent between species or when partitioning DA into tDA, pDA, and dDA. Given the very few number of studies and the considerable species and strain variability it is difficult to generalize from these experiments, particularly because some (Doucette et al., 2008; Pednekar et al., 2018) used fully-adapted cell cultures, Ayache et al. (2019) used abrupt salinity shifts, and our study was in between, with 2 transfers and gradual reduction in salinity. Bates (1998) suggested that DA may serve as an osmolyte, but none of these studies (Doucette et al., 2008; Ayache et al., 2019; Pednekar et al., 2018) could confirm that it is used as such. It is still possible that DA production is modified by energetic constraints (Doucette et al., 2008), but there was no statistically significant effect of salinity on growth in our study. If DA production were down-regulated to maintain high growth rates we would expect DA production and accumulation to decrease with decreasing salinity, which we did not observe. With regards to *Pseudo-nitzschia* and DA in SFB we conclude that salinity is not a barrier for maintenance of *Pseudo-nitzschia* in the system, as typical salinities for SFB (Fig. 8) would not suppress growth rates or toxin production. We do note however that at the lowest tested salinity the lag phase increased from a few days to ~12 days (Fig. 3B), suggesting that low salinity may act as a barrier to establishment of *Pseudo-nitzschia* in the fresher sub-embayments.

4.2. Temperature

Temperature has become a key parameter of interest in the regulation of *Pseudo-nitzschi*a blooms and toxicity along the U.S. west coast, because there is support for a correlation between warm oceanic periods driven by basin-scale oscillations (El Niño, Pacific Decadal Oscillation) and increased abundance and domoic acid accumulation for this region (McCabe et al., 2016; McKibben et al., 2017). The genus *Pseudo-nitzschia* exhibits a very wide range of temperature tolerance, from at least -1.5 °C to 30 °C (reviewed by Bates, 1998; Lelong et al., 2012). Earlier work identified a general trend of increasing DA with increasing temperature (Lewis et al., 1993; Bates et al., 1998), consistent with the assertion that warm events drive toxic blooms. This relationship appears to hold for the northern CCS but not the southern CCS. Sekula-Wood et al. (2011) found no relationship between *Pseudo-nitzschia* abundance, toxicity, and El Niño for a time series in the Santa Barbara Channel, while Barron et al. (2010) suggested anthropogenic activity on geological timescales in the Santa Barbara Basin is more closely linked to the emergence of the Santa Barbara Channel as a "hotspot" in the last few decades.

Conflicting evidence for the role of temperature in the southern CCS also emerges from comparison of field observations and experimental manipulations. Smith et al. (2018) compiled 15 years of field data and reported that significant DA was never recorded at temperatures > 19 °C. Ryan et al. (2017) similarly reported that peak toxicity in Monterey Bay during the 2015 Pacific warm anomaly was associated with a return to climatologically "cooler" conditions, when *P. australis* dominated (Bowers et al., 2018). In contrast, Zhu et al. (2017) isolated *P. australis* S7 from Southern California and reported that it did not begin to produce DA until 23 °C with maximum production at 30 °C, despite declining growth rate. This apparent contradiction could easily be explained by the small number of strains that have been tested, suggesting that warm-adapted variants of *P. australis* occur at background concentrations in the southern CCS and perhaps elsewhere but are not (currently) dominant. There are fewer studies of *P. multiseries*, but both Lewis et al. (1993) and Tatters et al. (2018) reported increasing toxicity with increasing temperature for this species.

Our results for growth are consistent with those reported by Zhu et al. (2017). As noted in that study, the McCabe et al. (2016) growth rates are considerably higher, but were conducted under much higher

light levels (250 µmol photons m^{-2} s^{-1}) compared to this study (120 µmol photons m^{-2} s^{-1}) and Zhu et al., 2017 (150 µmol photons m^{-2} s^{-1}). Despite the different temperature optima, calculated Q_{10} values from the three studies generally converged, with calculated values of 3.3–4.5 for 7–15 °C (McCabe et al. (2016), 3.3 (Zhu et al., 2017), and 2.73–3.09 (this study), comparable to a Q10 value of 2.11 for *P. multiseries* between 15–25 °C (Lewis et al., 1993). All studies therefore concluded that growth rate increases rapidly with warming until reaching a critical temperature between 20–30 °C depending on species and strain. During the 2015 Pacific Warm Anomaly, sea surface temperatures in the CCS were 4–5 °C above normal (Bond et al., 2015; Zaba and Rudnick, 2016). That increase in temperature would result in considerably enhanced growth for *Pseudo-nitzschia,* as well as a range shift towards more northern waters, since the warming would shift the northern CCS towards the temperature optima for *Pseudo-nitzschia*.

The critical question is what the upper thermal limit is for the species and strains occupying the CCS, and in particular the southern CCS. Our results are consistent with Smith et al. (2018) suggesting that above ~20–25 °C *Pseudo-nitzschia* will not be dominant in field assemblages. For SFB specifically, Peacock et al. (2018) reported that SFB exhibited mean temperatures of 16–20 °C for the 2015 water year with peak (record-breaking) temperatures in Central Bay of > 20 °C in August 2015. In Monterey Bay, peak bloom activity and toxicity occurred at temperatures of 10–15 °C (Ryan et al., 2017; Gentemann et al., 2017). In the Pacific Northwest, temperatures increased to 16-18° (McCabe et al., 2016), within the optimal thermal niche for *P. australis*, the dominant bloom-forming organism during 2015 (McCabe et al., 2017; Bowers et al., 2018).

Our results also suggest that maximum DA accumulation and production occurs at cool temperatures (10–15 °C), in contrast to *P. australis* S7 (Zhu et al., 2017). Our results are again consistent with field observations, where maximal DA was associated with cool (warm) waters in the southern (northern) CCS (McCabe et al., 2016; Ryan et al., 2017; Smith et al., 2018), with little to no DA at temperatures exceeding ~20 °C (Smith et al., 2018; Peacock et al., 2018). We also note that Lewis et al. (2018) observed a decrease in both cell density and domoic acid and an increase in the fraction of dDA for cells grown at 22 °C compared to 16 °C and 20 °C for *P. multiseries* CLNN-16 (isolated from Atlantic Canada). In contrast to most previous studies our results demonstrate decreasing DA accumulation and production with increasing temperature, and a corresponding decrease in the fraction of dDA in the tDA pool. This is again consistent with the compilation of field data for California (Smith et al., 2018) which documented virtually no pDA above 19 °C, and maximal pDA values between ~12–17 °C (dDA was not reported).

4.3. Domoic acid cell quotas and production

Cell quotas were variable but high compared to previous reports. *P. australis* has been reported to range from 0.026 to 37 pg $cell^{-1}$ in culture, while *P. multiseries* ranged from 0.021 to 67 pg $cell^{-1}$ (summarized in Trainer et al., 2012) with more recent values for *P. multiseries* reported to be as low as 10.9 fg cell (Martin-Jézéquel et al., 2015), compared to a maximum of 89.48 (± 34.30) and 124.59 (± 15.35) pg $cell^{-1}$ respectively for these experiments. While elevated, these values are consistent with field assemblages. For example, Ryan et al. (2017) reported maximum estimated values of 100 pg $cell^{-1}$ in Monterey Bay (CA) during the 2015 warm anomaly, Trainer et al., 2009a,b reported 63 pg $cell^{-1}$ in the Juan de Fuca eddy, Umhau et al. (2018) reported a maximum cellular DA of 1400 pg $cell^{-1}$ for the Santa Barbara Basin, and estimated cell quotas for Santa Cruz Municipal Wharf (Fig. 9) exceeded 100 pg $cell^{-1}$ (not including statistical outliers, which were much higher). Cell quotas were variable but not completely suppressed when using SFB18 or SFB33 base water (Fig. 4), suggesting that SFB water does not naturally completely inhibit domoic acid production. While there are relatively few measurements of both *Pseudo-nitzschia* and particulate toxin from natural assemblages of SFB, the maximum measured quota of 15.5 pg $cell^{-1}$ during 2015 suggests that native populations in SFB are capable of reaching typical toxin values observed in other systems and in culture.

Summarizing across all treatments, DA production varied considerably, with *P. multiseries* MW15190C3 ranging from 0 to 156.95 pg $cell^{-1}$ d^{-1} (median = 16.80, mean = 28.90 pg $cell^{-1}$ d^{-1}) while *P. australis* MLML-5 ranged from 0 to 106.96 pg $cell^{-1}$ d^{-1} (median = 18.01, mean = 24.07 pg $cell^{-1}$ d^{-1}). This is considerably higher than production rates for comparable CCS isolates. Zhu et al. (2017) reported a rate of ~7 pg cell d^{-1} for *P. australis* S7, while a natural assemblage of *Pseudo-nitzschia* on the Washington coast produced 0.075-0.080 pg $cell^{-1}$ d^{-1} (calculated using average growth rates and cell quotas from day 1 for nitrate, ammonium, and urea additions as reported in Radan and Cochlan, 2018). In contrast, calculated production rates from the natural assemblages dominated by *P. australis* in Monterey Bay during 2015 (McCabe et al., 2016) ranged from 13.11 to 38.15 pg $cell^{-1}$ d^{-1}, bracketing the median and mean values obtained for the isolates *P. australis* MLML-5 and *P. multseries* MW15190C3, the latter which was also isolated from Monterey Bay during the 2015 warm anomaly.

The effect of SFB base water differed for the two species. Toxin production increased considerably for *P. multiseries* MW15190C3 grown on SFB18 water (Table 3), while toxin production was suppressed but still comparable to other studies (~2 pg cell d^{-1}) for *P. australis* MLML-5 grown on both SFB18 and SFB33 base water. It is unclear what the specific mechanism(s) were for the effect of SFB water; there were not statistically significant differences with separate nutrient (ammonium, urea) additions, but adjusting salinity independently resulted in increases in DA production for both species, rather than suppression of DA production as was seen for *P. australis* MLML-5. Regardless of the mechanism the results again demonstrate that there is not consistent suppression of DA production in SFB water or as a response to manipulation of the salinity and nutrient composition. There is therefore no *a priori* reason to expect lower DA values for SFB compared to the open coast.

Very few studies have examined the relative partitioning of dDA and pDA. Based on Peacock et al. (2018), dDA is prevalent throughout SFB, even when pDA and mussel contamination are undetectable. Van Meerssche et al. (2018) found that dDA (and salinity) may have an allelopathic effect on other estuarine phytoplankton, but that study also reported conflicting correlations between dDA and salinity. *Pseudo-nitzschia* abundance and dDA were positively correlated, and both were correlated with increasing salinity in field observations, but there was a negative correlation with salinity for released DA in both field assemblages and bottle experiments. Umhau et al. (2018) reported strong correlations between pDA and dDA for natural assemblages in the Santa Barbara Basin and argued that dDA must have a similar half-life (months) in natural waters as pDA, arguing for the importance of dDA in the environment, and potentially explaining the ubiquity of dDA in SFB.

Maldonado et al. (2002) suggested that dDA was actively released from cells in response to metal stress as either iron limitation or excess copper. They speculated that DA may support a dual role in acquiring iron while detoxifying copper, although Lelong et al. (2012) reported that DA production in *P. multiseries* was not modified by copper exposure. In contrast, Fuentes and Wikfors (2013) reported synergistic effects between silicate and copper in *P. multiseries*, with both tDA and the fraction of dDA greatly enhanced with high silicate and high copper concentrations. In this study the percentage of dDA was substantially higher for the same salinity and with f/2 nutrients when comparing MLML, SFB18 and SFB33 base waters, with 62, 73, and 87% dDA respectively. With the addition of double EDTA the percentage dropped to 66% for SFB33 water. While not conclusive, these results are consistent with DA release from the cell acting as a chelator for metal(s) in the media that lengthened the lag phase for cells transferred into SFB33 water.

4.4. Increased lag time in San Francisco Bay water

While there were no consistent differences in growth, toxin production, or cell quotas for *P. multiseries* MW15190C3 and *P. australis* MLML-5 that would account for resistance of SFB to toxic blooms, significant increases in lag phase of growth were observed for several treatments. Specifically, lag time increased to ~10–12 days at very cold (10 °C) temperatures for both species, and also increased to ~11–12 days at salinity 18. For *P. australis* MLML-5 (*P. multiseries* was not tested), growth in 100% SFB33 base water also increased the lag time to 12 days (Fig. 3). This lag disappeared with the addition of extra EDTA, while no lag was evident (with or without extra EDTA) in MLML and SFB18 water. Chemical analysis beyond the basic parameters tested (Table 1) was not possible, but the dramatic effect of doubling the EDTA strongly suggests that some sort of trace metal inhibition was likely for the South Bay waters.

As discussed by Sahraoui et al. (2012), estuarine and lagoon systems such as Chesapeake Bay, parts of the Mediterranean, and the Gulf of Mexico may be particularly susceptible to toxic *Pseudo-nitzschia* blooms given typically elevated nutrients, long hydraulic retention times, and enclosed morphology. Toxic blooms of *Pseudo-nitzschia* are frequently associated with retentive regions (e.g. Anderson et al., 2006, 2011; Trainer et al., 2009a,b; Fawcett et al., 2007; Berdalet et al., 2017) including formation of thin layers (e.g. Rines et al., 2002, 2010; Velo-Suarez et al., 2008). Previous studies have documented the importance of wind events (Lucas et al., 2014; Louw et al., 2016) and tidal forcing on scales of hours to weeks in controlling *Pseudo-nitzschia* blooms (Díaz et al., 2014), suggesting that hydrological control of estuarine circulation may be key to bloom formation. Based on results for SFB, there is presumably interplay between physiological adaptation to changing coastal conditions, competition and hydrological residence time.

While every system is to some extent unique, it is instructive to compare the lack of toxic blooms in SFB with Chesapeake Bay, where domoic acid is frequently detected (Thessen and Stoecker, 2008) and is a serious threat (Thessen and Stoecker, 2008; Anderson et al., 2010). Although present year-round in Chesapeake Bay, abundance of *Pseudo-nitzschia* peaks seasonally with low temperatures and salinity greater than 10 (Thessen and Stoeker, 2010) suggesting that, similar to SFB, there is a specific set of environmental conditions leading to blooms. Unlike SFB, Chesapeake Bay has a very long residence time, averaging 180 days (Du and Shen, 2016), which is more than long enough for acclimation of resident or introduced populations of *Pseudo-nitzschia*. Thessen et al. (2005) reported very long lag phases in growth with step changes in salinity for *P. multiseries*, while Martin-Jézéquel et al. (2015) similarly reported longer lag phases for *P. multiseries* exposed to ammonium in culture experiments. Results from this study suggest prolonged acclimation is required when exposed to typical estuarine (elevated nutrients and metals, variable temperature and salinity) conditions.

4.5. Environmental variability as a regulator of Pseudo-nitzschia in SFB

Returning to the fundamental question as to why there are not more toxic *Pseudo-nitzschia* blooms in SFB, we hypothesize that typical environmental conditions would result in both an increase in the lag time for cells introduced from the coastal ocean in response to lowered salinity and exposure to copper or other inhibitory metals, while warmer temperatures would suppress toxin production. San Francisco Bay residence time is highly dependent on river flow and season, varying from 1 day to about two months, with longer residence times in South Bay and much shorter residence times in the central and northern basins (Cloern et al., 2017). Given a 10–12 day lag in growth it is reasonable to assume that a combination of salinity and chemical inhibitors could keep coastal *Pseudo-nitzschia* from establishing a resident population. This is also consistent with microscopy (Nejad et al., 2017), with *Pseudo-nitzschia* spp. very common in Central Bay (direct exchange with the coastal ocean), less common in South Bay (inhibition by metals), and fairly rarely observed in the northern (low salinity, shorter residence time) bays. In our study *Pseudo-nitzschia* was eventually able to adapt to changing conditions (temperature, salinity, base water) but in SFB it is presumably fairly rare for a given water mass to retain its physical and chemical characteristics for the 10–12 days that it takes for these strains to adapt in culture.

Berg et al. (2017) recently compared growth rate of several common phytoplankton genera isolated from SFB and grown under comparable conditions (SFB water amended with f/2 media at similar irradiance) as this study. Growth rates varied from ~0.4 – 1.0 d^{-1} depending on nitrogen source and concentration. At 15 °C (the temperature used in Berg et al. 2016), *Pseudo-nitzschia* growth rates were comparable to but lower than the diatoms *Entomoneis paludosa*, *Thalassiosira weissflogii*, *Asterionella ralfsii*, and *Fragilaria capucina* grown on NO_3-, suggesting that *Pseudo-nitzschia* might also simply be out-competed at moderate temperatures within SFB. As temperatures increase our results suggest that toxin production would also decline, resulting in *Pseudo-nitzschia* remaining a minor component of the overall diatom assemblage while tDA decreased. Combined with increasing lag time for *Pseudo-nitzschia* in response to estuarine conditions it is reasonable to conclude that *Pseudo-nitzschia* is simply not the most competitive organism under typical historical conditions within SFB. This is again consistent with observations from the USGS time-series, which document occasional increases in *Pseudo-nitzchia* abundance but no widespread blooms (Nejad et al., 2017). We suggest that *Pseudo-nitzschia* is capable of growing in a wide range of coastal/estuarine conditions but that a primary limitation on bloom formation in estuaries is related to this prolonged acclimation period and intrinsic growth rates (physiological control) versus retention (hydrological control).

4.6. Summary

Two representative coastal isolates of *Pseudo-nitzschia* exhibited reasonable growth rates and comparable or enhanced toxicity compared to growth and toxicity of the same species grown with coastal base water. There does not appear to be any one environmental factor limiting the occurrence of toxic *Pseudo-nitzschia* blooms in SFB. *Pseudo-nitzschia* exhibits broad tolerance to both temperature and salinity and is not uncommon in SFB, but a combination of estuarine circulation, lower salinity, chemical inhibition, and competition with other resident diatoms may preclude establishment of a resident population due to insufficient time for advected populations to adapt to estuarine conditions. The warm dry period characteristic of SFB in 2015 resulted in a significant increase in DA in resident mussels, and those conditions would enhance growth rates and prolong potential residence time of *Pseudo-nitzschia* in the Bay. However, this shift in conditions was apparently insufficient to allow *Pseudo-nitzschia* to become dominant in SFB waters, while the warm temperatures likely suppressed toxin production which led to much lower overall toxicity compared to the open coast.

The Bay's historical resilience to blooms may change in the future (Sutula et al., 2017), and based on our results, given sufficient time to adapt, *Pseudo-nitzschia* is very capable of maintaining high growth rates within SFB with enhanced toxicity from lower salinity and exposure to the chemical makeup of SFB water. Despite the historical resilience there is still good evidence for routine exchange of cells and/or toxin with the coastal ocean (Peacock et al., 2018) providing SFB with a consistent source population of *Pseudo-nitzschia* to grow and produce considerable amounts of toxin. It would therefore be unwise to assume that SFB will maintain its resilience, particularly if more warm-adapted species (Zhu et al., 2017) emerge from the background population at some point in the future.

Author contributions

R.M.K. and K.H. designed the study. C.G. performed *Pseudo-nitschia* quantification, and all authors participated in data collection and analysis. R.M.K. wrote the paper. All authors contributed to editing and revisions.

Competing interests

The authors declare that they have no financial or commercial conflicts of interest.

Acknowledgments

We thank Erica Nejad and Dana Shultz for field collection of San Francisco Bay water used in this study as part of the USGS long-term monitoring program, and H. Bowers and A. Woods for the cell cultures. The conceptual basis for this analysis developed from discussions with the Nutrients as Numeric Endpoints program supported by the San Francisco Estuary Institute (SFEI), and we thank Jim Cloern, Dave Senn, and their teams for fruitful discussions and feedback. The initial version of this manuscript was greatly improved with comments from two anonymous reviewers. This work was supported by the National Oceanic and Atmospheric Administration (NA11NOS4780030) through the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program, publication number ECO931.[CG]

References

Alpine, A.E., Cloern, J.E., 1988. Phytoplankton growth rates in a light-limited environment, San Francisco Bay. Mar. Ecol. Prog. Ser. 44, 167–173.
Anderson, C.R., Brzezinski, M.A., Washburn, L., Kudela, R., 2006. Circulation and environmental conditions during a toxigenic Pseudo-nitzschia australis bloom in the Santa Barbara Channel, California. Mar. Ecol. 327, 119–133.
Anderson, C.R., Sapiano, M.R., Prasad, M.B.K., Long, W., Tango, P.J., Brown, C.W., Murtugudde, R., 2010. Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay. J. Mar. Syst. 83, 127–140.
Anderson, C.R., Kudela, R.M., Benitez-Nelson, C., Sekula-Wood, E., Burrell, C.T., Chao, Y., Langlois, G., Goodman, J., Siegel, D.A., 2011. Detecting toxic diatom blooms from ocean color and a regional ocean model. Geophys. Res. Lett. 38 (4).
Auro, M.E., Cochlan, W.P., 2013. Nitrogen utilization and toxin production by two diatoms of the *Pseudo-nitzschia pseudodelicatissima* Complex: *P. cuspidata* and *P. fryxelliana*. J. Phycol. 49, 156–169.
Ayache, N., Hervé, F., Martin-Jézéquel, V., Amzil, Z., Caruana, A.M., 2019. Influence of sudden salinity variation on the physiology and domoic acid production by two strains of Pseudoénitzschia australis. J. Phycol. 55 (1), 186–195.
Barron, J.A., Bukry, D., Field, D., 2010. Santa Barbara Basin diatom and silicoflagellate response to global climate anomalies during the past 2200 years. Quat. Int. 215, 34–44.
Bates, S.S., 1998. Ecophysiology and metabolism of ASP toxin production. NATO ASI Ser. G Ecol. Sci. 41, 405–426.
Bates, S.S., Garrison, D.L., Horner, R.A., 1998. Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. NATO ASI Ser. G Ecol. Sci. 41, 267–292.
Bates, S.S., Hubbard, K.A., Lundholm, N., Montresor, M., Leaw, C.P., 2018. *Pseudo-nitzschia, Nitzschia*, and domoic acid: new research since 2011. Harmful Algae 79, 3–43.
Berdalet, E., Montresor, M., Reguera, B., Roy, S., Yamazaki, H., Cembella, A., Raine, R., 2017. Harmful algal blooms in fjords, coastal embayments, and stratified systems: recent progress and future research. Oceanography 30, 46–57.
Berg, G.M., Driscoll, S., Hayashi, K., Ross, M., Kudela, R., 2017. Variation in growth rate, carbon assimilation, and photosynthetic efficiency in response to nitrogen source and concentration in phytoplankton isolated from upper San Francisco Bay. J. Phycol. 53, 664–679.
Bond, N.A., Cronin, M.F., Freeland, H., Mantua, N., 2015. Causes and impacts of the 2014 warm anomaly in the NE Pacific. Geophys. Res. Lett. 42, 3414–3420.
Bouillon, R.C., Knierim, T.L., Kieber, R.J., Skrabal, S.A., Wright, J.L., 2006. Photodegradation of the algal toxin domoic acid in natural water matrices. Limnol. Oceanogr. 51, 321–330.
Bowers, H.A., Ryan, J.P., Hayashi, K., Woods, A.L., Marin III, R., Smith, G.J., Hubbard, K.A., Doucette, G.J., Mikulski, C.M., Gellene, A.G., 2018. Diversity and toxicity of *Pseudo-nitzschia* species in Monterey Bay: perspectives from targeted and adaptive sampling. Harmful Algae 78, 129–141.
Chaui-Berlinck, J.G., Monteiro, L.H.A., Navas, C.A., Bicudo, J.E.P., 2002. Temperature effects on energy metabolism: a dynamic system analysis. Proc. R. Soc. Lond. B Biol. Sci. 269, 15–19.
Cloern, J.E., 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Rev. Geophys. 34, 127–168.
Cloern, J.E., 2019. Patterns, pace, and processes of water-quality variability in a long--studied estuary. Limnol Oceanogr. 64 (S1), S192–S208.
Cloern, J.E., Dufford, R., 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. Mar. Ecol. Prog. Ser. 285, 11–28.
Cloern, J.E., Jassby, A.D., 2012. Drivers of change in estuarine-coastal ecosystems: discoveries from four decades of study in San Francisco Bay. Rev. Geophys. 50. https://doi.org/10.1029/2012RG000397.
Cloern, J.E., Jassby, A.D., Schraga, T.S., Nejad, E., Martin, C., 2017. Ecosystem variability along the estuarine salinity gradient: examples from long-term study of San Francisco Bay. Limnol. Oceanogr. 62. https://doi.org/10.1002/lno.10537.
Cloern, J.E., Schraga, T.S., 2016. USGS Measurements of Water Quality in San Francisco Bay (CA), 1969-2015 (ver.3.0 June 2017): U.S. Geological Survey Data Release. https://doi.org/10.5066/F7TQ5ZPR.
Cochlan, W.P., Herndon, J., Kudela, R.M., 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). Harmful Algae 8, 111–118.
Díaz, P.A., Ruiz-Villarreal, M., Velo-Suárez, L., Ramilo, I., Gentien, P., Lunven, M., Fernand, L., Raine, R., Reguera, B., 2014. Tidal and wind-event variability and the distribution of two groups of *Pseudo-nitzschia* species in an upwelling-influenced Ría. Deep Sea Res. Part II 101, 163–179.
Doucette, G.J., King, K.L., Thessen, A.E., Dortch, Q., 2008. The effect of salinity on domoic acid production by the diatom *Pseudo-nitzschia multiseries*. Nova Hedwigia 133, 31–46.
Du, J., Shen, J., 2016. Water residence time in Chesapeake Bay for 1980–2012. J. Mar. Syst. 164, 101–111.
Fawcett, A., Pitcher, G.C., Bernard, S., Cembella, A.D., Kudela, R.M., 2007. Contrasting wind patterns and toxigenic phytoplankton in the southern Benguela upwelling system. Mar. Ecol. 348, 19–31.
Fuentes, M.S., Wikfors, G.H., 2013. Control of domoic acid toxin expression in *Pseudo-nitzschia multiseries* by copper and silica: relevance to mussel aquaculture in New England (USA). Mar. Environ. Res. 83, 23–28.
Gentemann, C.L., Fewings, M.R., García Reyes, M., 2017. Satellite sea surface temperatures along the West Coast of the United States during the 2014–2016 northeast Pacific marine heat wave. Geophys. Res. Lett. 44, 312–319.
Hagström, J.A., Granéli, E., Maneiro, I., Barreiro, A., Petermann, A., Svensen, C., 2007. Release and degradation of amnesic shellfish poison from decaying *Pseudo-nitzschia* multiseries in presence of bacteria and organic matter. Harmful Algae 6, 175–188.
Holmes, R.M., Aminot, A., Kérouel, R., Hooker, B.A., Peterson, B.J., 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56, 1801–1808.
Howard, M.D.A., Cochlan, W.P., Ladizinsky, N., Kudela, R.M., 2007. Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. Harmful Algae 6, 206–217.
Knepel, K., Bogren, K., 2001. Determination of Orthophosphorous by Flow Injection Analysis in Seawaters: QuickChem Method 31-115-01-1-H. Saline Methods of Analysis, Lachat Instruments, Milwaukee, WI.
Kudela, R., Roberts, A., Armstrong, M., 2004. Laboratory analyses of nutrient stress and toxin accumulation in *Pseudonitzschia* species from Monterey Bay, California. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris, pp. 136–138.
Kudela, R.M., Lane, J.Q., Cochlan, W.P., 2008. The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA. Harmful Algae 8, 103–110.
Kudela, R.M., Seeyave, S., Cochlan, W.P., 2010. The role of nutrients in regulation and promotion of harmful algal blooms in upwelling systems. Prog. Oceanogr. 85, 122–135.
Lelong, A., Hégaret, H., Soudant, P., Bates, S.S., 2012. *Pseudo-nitzschia* (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms. Phycologia 51, 168–216.
Lewis, N.I., Bates, S.S., McLachlan, J.L., Smith, J.C., 1993. Temperature effects on growth, domoic acid production, and morphology of the diatom *Nitzschia pungens f. multiseries*. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Science Publishers B.V., pp. 601–606.
Lewis, N.I., Bates, S.S., Quilliam, M.A., 2018. Production of domoic acid from large-scale cultures of *Pseudo-nitzschia multiseries*: a feasibility study. Harmful Algae 79, 58–63.
Lewitus, A.J., Horner, R.A., Caron, D.A., Garcia-Mendoza, E., Hickey, B.M., Hunter, M., Huppert, D.D., Kudela, R.M., Langlois, G.W., Largier, J.L., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. Harmful Algae 19, 133–159.
Louw, D.C., Doucette, G.J., Voges, E., 2016. Annual patterns, distribution and long-term trends of *Pseudo-nitzschia* species in the northern Benguela upwelling system. J. Plankton Res. 39, 35–47.
Lucas, A.J., Pitcher, G.C., Probyn, T.A., Kudela, R.M., 2014. The influence of diurnal winds on phytoplankton dynamics in a coastal upwelling system off southwestern Africa. Deep Sea Res. Part II 101, 50–62.
Maldonado, M.T., Hughes, M.P., Rue, E.L., Wells, M.L., 2002. The effect of Fe and Cu on growth and domoic acid production by Pseudo-nitzschia multiseries and *Pseudo-nitzschia australis*. Limnol. Oceanogr. 47, 515–526.
Martin-Jézéquel, V., Calu, G., Candela, L., Amzil, Z., Jauffrais, T., Séchet, V., Weigel, P., 2015. Effects of organic and inorganic nitrogen on the growth and production of domoic acid by *Pseudo-nitzschia multiseries* and *P. australis* (Bacillariophyceae) in culture. Mar. Drugs 13, 7067–7086.
McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D.,

Gulland, F., Thomson, R.E., Cochlan, W.P., Trainer, V.L., 2016. An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. Geophys. Res. Lett. 43. https://doi.org/10.1002/2016GL070023.

McKibben, S.M., Peterson, W., Wood, A.M., Trainer, V.L., Hunter, M., White, A.E., 2017. Climatic regulation of the neurotoxin domoic acid. Proc. Natl. Acad. Sci. 114, 239–244.

Mengelt, C., Prézelin, B.B., 2002. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A. (Eds.), Dark Survival and Subsequent Light Recovery for Pseudo-nitzschia Multiseries. Harmful Algae, pp. 388–390.

Mulvenna, P.F., Savidge, G., 1992. A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent. Estuar. Coast. Shelf Sci. 34, 429–438.

Nejad, E.S., Schraga, T.S., Cloern, J.E., 2018. Phytoplankton Species Composition, Abundance and Cell Size in San Francisco Bay: Microscopic Analyses of USGS Samples, Beginning in 2014: U.S. Geological Survey Data Release. https://doi.org/10.5066/F7C24VB2.

Nejad, E.S., Schraga, T.S., Cloern, J.E., 2017. Phytoplankton Species Composition, Abundance and Cell Size in San Francisco Bay: Microscopic Analyses of USGS Samples Collected 1992-2014: U.S. Geological Survey Data Release. https://doi.org/10.5066/F74F1P6P.

Peacock, M.B., Gibble, C.M., Senn, D.B., Cloern, J.E., Kudela, R.M., 2018. Blurred lines: multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California. Harmful Algae 73, 138–147.

Pednekar, M., Bates, S.S., Kerkar, V., Matondkar, S., 2018. Environmental factors affecting the distribution of *Pseudo-nitzschia* in two monsoonal estuaries of western India and effects of salinity on growth and domoic acid production by *P. pungens*. Estuaries Coasts 41, 1448–1462.

Price, N.M., Harrison, P.J., 1987. Comparison of methods for the analysis of dissolved urea in seawater. Mar. Biol. 94, 307–317.

Radan, R.L., Cochlan, W.P., 2018. Differential toxin response of *Pseudo-nitzschia multiseries* as a function of nitrogen speciation in batch and continuous cultures, and during a natural assemblage experiment. Harmful Algae 73, 12–29.

Rines, J.E.B., Donaghay, P.L., Dekshenieks, M.M., Sullivan, J.M., Twardowski, M.S., 2002. Thin layers and camouflage: hidden *Pseudo-nitzschia* spp.(Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. Mar. Ecol. Prog. Ser. 225, 123–137.

Rines, J.E.B., McFarland, M.N., Donaghay, P.L., Sullivan, J.M., 2010. Thin layers and species-specific characterization of the phytoplankton community in Monterey Bay, California, USA. Cont. Shelf Res. 30, 66–80.

Ryan, J.P., Kudela, R.M., Birch, J.M., Blum, M., Bowers, H.A., Chavez, F.P., Doucette, G.J., Hayashi, K., Marin, R., Mikulski, C.M., 2017. Causality of an extreme harmful algal bloom in Monterey Bay, California, during the 2014–2016 northeast Pacific warm anomaly. Geophys. Res. Lett. 44, 5571–5579.

Sahraoui, I., Grami, B., Bates, S.S., Bouchouicha, D., Chikhaoui, M.A., Mabrouk, H.H., Hlaili, A.S., 2012. Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) populations and domoic acid to environmental conditions in a eutrophied, SW Mediterranean coastal lagoon (Tunisia). Estuar. Coast. Shelf Sci. 102, 95–104.

Schraga, T.S., Nejad, E.S., Martin, C.A., Cloern, J.E., 2018. USGS Measurements of Water Quality in San Francisco Bay (CA), Beginning in 2016 (ver.2.0, June 2018): U.S. Geological Survey Data Release. https://doi.org/10.5066/F7D21WGF.

Sekula-Wood, E., Benitez-Nelson, C., Morton, S., Anderson, C., Burrell, C., Thunell, R., 2011. *Pseudo-nitzschia* and domoic acid fluxes in Santa Barbara Basin (CA) from 1993 to 2008. Harmful Algae 10, 567–575.

Smith, J., Connell, P., Evans, R.H., Gellene, A.G., Howard, M.D., Jones, B.H., Kaveggia, S., Palmer, L., Schnetzer, A., Seegers, B.N., 2018. A decade and a half of *Pseudo-nitzschia* spp. and domoic acid along the coast of southern California. Harmful Algae 79, 87–104.

Smith, P., Bogren, K., 2001. Determination of Silicate in Brackish or Seawater by Flow Injection Analysis Colorimeter: QuickChem Method 31-114-27-1-C. Saline Methods of Analysis, Lachat Instruments, Milwaukee, WI.

Sutula, M., Kudela, R., Hagy III, J.D., Harding Jr, L.W., Senn, D., Cloern, J.E., Bricker, S., Berg, G.M., Beck, M., 2017. Novel analyses of long-term data provide a scientific basis for chlorophyll-a thresholds in San Francisco Bay. Estuar. Coast. Shelf Sci. 197, 107–118.

Tatters, A.O., Schnetzer, A., Xu, K., Walworth, N.G., Fu, F., Spackeen, J.L., Sipler, R.E., Bertrand, E.M., McQuaid, J.B., Allen, A.E., 2018. Interactive effects of temperature, CO_2 and nitrogen source on a coastal California diatom assemblage. J. Plankton Res. 40, 151–164.

Thessen, A.E., Dortch, Q., Parsons, M.L., Morrison, W., 2005. Effect of salinity on *Pseudo-nitzschia* species (Bacillariophceae) growth and distribution. J. Phycol. 41, 21–29.

Thessen, A.E., Stoecker, D.K., 2008. Distribution, abundance and domoic acid analysis of the toxic diatom genus *Pseudo-nitzschia* from the Chesapeake Bay. Estuaries Coasts 31, 664–672.

Tjørve, K.M., Tjørve, E., 2017. The use of Gompertz models in growth analyses, and new Gompertz-model approach: an addition to the Unified-Richards family. PLoS One 12, e0178691.

Trainer, V.L., Adams, N.G., Bill, B.D., Anulacion, B.F., Wekell, J.C., 1998. Concentration and dispersal of a *Pseudo-nitzschia*bloom in Penn Cove, Washington, USA. Nat. Toxins 6, 113–125.

Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., Trick, C.G., 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14, 271–300.

Trainer, V.L., Cochlan, W.P., Erickson, A., Bill, B.D., Cox, F.H., Borchert, J.A., Lefebvre, K.A., 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae 6, 449–459.

Trainer, V.L., Hickey, B.M., Lessard, E.J., Cochlan, W.P., Trick, C.G., Wells, M.L., MacFadyen, A., Moore, S.K., 2009a. Variability of *Pseudo-nitzschia* and domoic acid in the Juan de Fuca eddy region and its adjacent shelves. Limnol. Oceanogr. 54, 289–308.

Trainer, V.L., Wells, M.L., Cochlan, W.P., Trick, C.G., Bill, B.D., Baugh, K.A., Beall, B.F., Herndon, J., Lundholm, N., 2009b. An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington State coast. Limnol. Oceanogr. 54, 1461–1474.

Umhau, B., Benitez-Nelson, C., Anderson, C., McCabe, K., Burrell, C., 2018. A time series of water column distributions and sinking particle flux of *Pseudo-nitzschia* and domoic acid in the Santa Barbara Basin, California. Toxins 10, 480.

Van Meerssche, E., Greenfield, D.I., Pinckney, J.L., 2018. Coastal eutrophication and freshening: impacts on Pseudo-nitzschia abundance and domoic acid allelopathy. Estuar. Coast. Shelf Sci. 209, 70–79.

Velo-Suarez, L., Gonzalez-Gil, S., Gentien, P., Lunven, M., Bechemin, C., Fernand, L., Raine, R., Reguera, B., 2008. Thin layers of *Pseudo-nitzschia* spp. and the fate of *Dinophysis acuminata* during an upwelling–downwelling cycle in a Galician Ria. Limnol. Oceanogr. 53, 1816–1834.

Wang, Z., King, K.L., Ramsdell, J.S., Doucette, G.J., 2007. Determination of domoic acid in seawater and phytoplankton by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1163, 169–176.

Wolters, M., 2002. Determination of silicate in brackish or seawater by flow injection analysis. QuikChemH Method 31–114.

Zaba, K.D., Rudnick, D.L., 2016. The 2014–2015 warming anomaly in the Southern California Current System observed by underwater gliders. Geophys. Res. Lett. 43, 1241–1248.

Zhu, Z., Qu, P., Fu, F., Tennenbaum, N., Tatters, A.O., Hutchins, D.A., 2017. Understanding the blob bloom: warming increases toxicity and abundance of the harmful bloom diatom *Pseudo-nitzschia* in California coastal waters. Harmful Algae 67, 36–43.