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Inter and intra-specific growth and domoic acid production in relation to nutrient ratios and concentrations in *Pseudo-nitzschia* : phosphate an important factor

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Abstract :

The factors responsible for inducing the synthesis of toxins and responses from toxic phytoplankton blooms remain unclear. In this study we compare the influence of genotypic (at both the intra and interspecific levels) and environmental factors (nutrient concentration and ratio) on growth (in terms of cell densities) and domoic acid (DA) production in three *Pseudo-nitzschia* species: *P. australis, P.pungens* and *P.fradulenta*. A strong phosphate effect was detected. More precisely, a low initial concentration in phosphate, even at high initial nitrogen and silicate concentrations, induced the highest DA concentrations and the lowest cell densities in all strains/species studied. In contrast, a low initial concentration of nitrogen and silicate combined, with a higher phosphate concentration resulted in low cell densities, but without high DA production. Inter-species effects were also observed in DA production, where *P. australis* represented the most toxigenic species of all. Intra-specific variations were only moderate, except for a recently isolated *P. australis* strain, suggesting the influence of time since isolation on the physiology and DA production of *Pseudo-nitzschia* species. Overall, the lack of strong interaction between environmental and genotypic factors showed that the various genotypes investigated did not extensively diverge in their ability to respond (in terms of DA production and cell densities) to contrasting nutrient supply.

Keywords : *Pseudo-nitzschia* strains, Domoic acid, Ratio, Phosphate, Nitrogen

1. Introduction

Within the pelagic ecosystem, the synthesis of toxic secondary metabolites, such as domoic acid by *Pseudo-nitzschia* species, and their accumulation in the trophic networks generate health risks, especially for consumers of shellfish (reviewed in Trainer et al., 2012). *Pseudo-nitzschia* are cosmopolitan diatoms that

include forty-eight species with 23 of them recently listed to produce domoic acid (DA) (Teng et al, 2016; Zabaglo et al., 2016), a toxin responsible for amnesic poisoning following the consumption of contaminated shellfish (reviewed in Trainer et al., 2012 and Lelong et al., 2012). This amino acid is an analogue of a neurotransmitter (glutamic acid) and can cause, in humans and other animals (e.g. seals and birds), gastrointestinal disorders followed by neurological problems that can lead to death (La Barre et al., 2014). Moreover, these toxic events have significant socio-economic impacts on shellfish industries and fisheries (Belin et al., 2013). However, the factors responsible for inducing the synthesis of these toxins remain unclear (reviewed in Lelong et al., 2012; Trainer et al., 2012; Zabaglo et al., 2016).

Domoic acid synthesis is governed by several biotic and abiotic factors. Genotypic differences (i.e inter and intra species differences), life stages, biological interactions with bacteria, and abiotic environmental parameters have all appeared to influence DA production (e.g. Bates et al., 1998; Maldonado et al., 2002; Kaczmarska et al., 2005; Thesssen et al., 2009; Amato et al., 2010; Guannel et al., 2011; Lelong et al., 2013; Lundholm et al., 2012; Sison-Mangus et al., 2014; Martin-Jézéquel et al., 2015). Inter-specific differences are inferred from the fact that some species, such as *Pseudo-nitzschia australis* can produce higher amounts of DA than other species in particular *P.pungens* and *P.fraudulenta* (reviewed in Trainer et al., 2012). There also seems to be strong intra-specific variability, but it is a relatively unexplored domain (Thessen et al., 2009). Several studies have looked at the influence of environmental parameters on toxin production, mainly focusing on the impact of diverse nitrogen sources (e.g. mineral nitrogen NO_3^- and organic nitrogen such as urea (reviewed in Martin-Jézéquel et al., 2015) and of limitation of different elements such as phosphate, silicate, nitrogen and iron (Bates et al., 1991; Pan et al., 1996a, 1996b;

78 Maldonado et al., 2002; Fehling et al., 2004; Lelong et al., 2013). While all these
79 factors seem to strongly influence the production of DA, understanding the
80 phenomenon remains very fragmented and similar studies have found different results
81 in different parts of the world, (Bates 1998; Bates and Trainer 2006).
82 Redfield (1934) described that phytoplankton and seawater across the oceans
83 have a similar atomic ratio of elements of 106:16:1 (C:N:P), suggesting that the ratio
84 between nutrients is as important as their concentration, and he defined a
85 stoichiometric equilibrium of elements. Later, Brzezinski (1985) expanded Redfield's
86 ratio of nutrients specifically for Diatoms and included silicate (Si:N:P = 15:16:1
87 ratio).While it is likely more complex than previously believed, as there are
88 substantial elemental variations among ocean taxa and environmental changes, this
89 ratio is the most broadly applied stoichiometric reference for nutrient limitation of
90 planktonic production (Martiny et al., 2016).
91 Previous studies on different *Pseudo-nitzschia* species showed that, when
92 nitrogen is available, a limitation in silicate or phosphate induced an increase in DA
93 production in cultures (Bates et al., 1991, 1998; Pan et al., 1996a, 1996b; Fehling et
94 al., 2004). However, such studies generally only look at one nutrient parameter at a
95 time and disregard the fate of the other nutrients. Indeed, there seems to be a lack of
96 attention towards the effect of different N:P:Si ratios and of the limitation of one
97 nutrient versus the other. Particularly, there are few studies looking at the effect of
98 Phosphate availability and ratio. On another note, only a handful of studies have
99 looked at intra-specific differences between different strains of DA producing diatoms
100 (e.g. Thessen et al., 2009; Hagström et al, 2010). Together, these factors could explain
101 part of the variability in studies looking at DA production.

In the present study, we assayed the growth (in terms of cell densities at stationary phase) and DA production of nine strains from three toxigenic *Pseudo-nitzschia* species (*P. australis*, *P. pungens*, *P. fraudulenta*) in relation to phosphate availability, considering different Si:N:P ratios (in accordance or deviating from the Redfield-Brzezinski's stoichiometric Si:N:P = 15:16:1 ratio) (Redfield, 1934; Brzezinski 1985). Our goal was to decipher at what point *Pseudo-nitzschia* phenotypic responses (cell densities and DA production) were driven by genotypes, at both intra- and inter-specific levels, and/or environmental factors (phosphate availability).

2. MATERIALS AND METHODS

2.1. Collection, identification and maintenance of Pseudo-nitzschia *cultures*

Strains of three *Pseudo-nitzschia* species (*P. australis*, *P. pungens* and *P. fraudulenta*) were collected and isolated from different locations on the north coast of France (Normandy, English Channel) and the West Finistère coast of France (Brittany, Atlantic coast). For each species, three strains (nine strains in total) were selected for this study (Table 1).

To establish monoclonal strains, single cells were isolated using a sterile micropipette, washed three times with filter-sterilized seawater and transferred to K/2 + Si culture medium (Keller et al., 1987). *Pseudo-nitzschia* species and strains (Table 1) were identified using TEM and through sequencing of the gene ITS1-5, 8S-ITS2. ITS1 and ITS2 regions were extracted from large genome data sets for all strains (undergoing project). And all the strains from a given species had identical sequences (GenBank accession numbers KY317919-KY317921).

126 Cultures were maintained in sterile-filtered oligotrophic seawater amended with
127 K/2 + Si medium (100.8 µM $Na_2SiO_3.5H_2O$; pH ~8; salinity ~33.5) (modified from
128 Keller et al., 1987) at 16 °C and under a 12:12 L:D cycle and 80 µmol.photons.$m^{-2}.s^{-1}$
129 in an algal incubator.
130 For cell size measurements, each strain was observed under a Nikon Eclipse 80i
131 light microscope equipped with a Nikon DS-Ri2 camera and 20 cells of each strain
132 were measured (length and width) using NIS-Elements Imaging Software. The strain
133 size was calculated as the mean ± standard deviation of 20 cells measured.
134
135 *2.2. Experimental setup: varying the initial Si:N:P ratio*
136 To test the effect of initial phosphate concentration and ratio (i.e. in relation to
137 coupled nitrate and silicate availability) on cell densities and DA concentrations, three
138 strains from each of the 3 studied species (making a total of 9 strains; see Table 1)
139 were grown in nine modified K/2 + Si media, in which concentrations of $NaNO_3$,
140 $NaH_2PO_4.H_2O$ and $Na_2SiO_3.5H_2O$ varied (at 12:12 L:D cycle, temperature ~16 °C
141 and pH ~8) (nb: no NH_4 was added, in order to have only one source of nitrogen for
142 the experiments). In our study we were primarily interested in observing the effects of
143 phosphate availability in comparison to N and Si. Therefore, similarly to Redfield-
144 Brezinsky stoichiometric ratio (C:Si:N:P = 106:15:16:1) initial silicate concentrations
145 were set to vary along with the variation of nitrogen (1:1 ratio). Three media consisted
146 of initial Si:N:P =16:16:1 at increasing concentrations (i.e. media M1, M5 and M9).
147 The other six (M2, M3, M4, M6, M7 and M8) were set to deviate from the Redfield-
148 Brezinsky ratio by having phosphate and/or nitrate and silicate in excess or in
149 limitation, as presented in Figure 1. Since initial nitrate and silicate concentrations
150 were equivalent in all media, they will be referred in the text as “N_Si”.

151 All strains and species were grown in triplicate for each of the nine
152 experimental media, in 40-ml flasks (T25, Sarstedt AG & Co, Nümbrecht, Germany)
153 containing 20 ml and a starting cell concentration of ~2000 cells.ml^{-1} (making a total
154 of 243 cultures). In order to collect cells at stationary phase, growth was followed by
155 subsampling 1 ml of homogenized culture every two days into a 48-well plate and
156 quantifying *in vivo* chlorophyll fluorescence (ex: 440/40, em: 680/20) in a FLX800
157 fluorescence microplate reader (Biotek Inc., VT, USA). Samples were collected at
158 stationary phase, 4 to 9 days after reaching maximum fluorescence (see
159 Supplementary Fig. 1 and Supplementary Table 1 to visualize growth trends and dates
160 of collection for each sample).
161 At late stationary phase, samples (all cells plus medium in the flask) were
162 collected, snap frozen in liquid nitrogen and stored at -80 °C. Subsamples were also
163 fixed in Lugol's iodine (Merck, Darmstadt, Germany) for cell counts, using a Heimm
164 cell counter and a light microscope (Olympus BX51, Japan).
165
166 *2.3 Domoic acid quantification*
167 Stored cultures were thawed and centrifuged at ~2,000g for 20 min to separate
168 cells from the medium and therefore to distinguish particulate DA (PDA) from
169 dissolved DA (DDA).
170 Particulate DA was extracted (from centrifuged *Pseudo-nitzschia* sp. cells) by adding
171 200 µl of 10% methanol, sonicating on ice for 90 s (Ultrasonic processor, Sonics &
172 Materials Inc., CT, USA) and centrifuging for 5 min at ~18,000g (Litaker et al.,
173 2008). DA concentration was analyzed using a DA ELISA kit (Mercury Science,
174 Durham, NC, USA), following the manufacturer's instructions, and measured on a

175 spectrophotometer microplate reader (Multiskan Ex, Thermo Electron Corp., Vantaa,
176 Finland).
177 For DDA, no extraction was required and samples were processed directly with the
178 Mercury Science DA kit, as per the manufacturer's instructions.
179 DA concentrations were calculated from absorbance (OD) using the
180 manufacturer's procedures and formulas. The detection limit was 0.01 ppb DA($\mu g.L^{-1}$)
181 in the extraction buffer.
182
183 *2.4. Statistics analysis*
184 Media were assigned to different groups corresponding to their phosphate and
185 N_Si concentration, as represented in Figure 1. Phosphate and N_Si concentrations
186 were divided into three categories: low, medium and high (Fig. 1). An alternative to
187 this nutrient level categorization would have been to categorize the media in term of
188 initial deviation from the Redfield-Brezinsky ratio. However, the exploratory
189 visualization of the results pointed towards a strong effect of nutrient levels and a
190 weak effect of initial ratios. As nutrient levels and initial ratios are not independent,
191 only nutrient levels were used as explanatory variables.
192 Cell density measurements were based on: 1) cell abundance for a single
193 replicate and 2) end-point fluorescence units for all replicates (see above); both
194 collected at stationary phase (i.e. final time point). In accordance to these units of
195 growth, DA was defined as: 1) $DA.cell^{-1}$ and 2) DA per unit of fluorescence
196 ($DA.fluorescence^{-1}$).
197 The effects of phosphate and N_Si levels, species and strain factors on both
198 cell density and DA production (total and particulate) were analyzed using three
199 ANOVA linear models (aov function in R package version 3.2.4).

In the first model, using final cell counts as a proxy for growth (collected ~ 4 to 9 days after reaching maximum cell concentration), and quantifying DA per cell (n=81, one measure per strain and per medium, the strains being considered as replicates within the species): the effect of Species, P levels, N_Si levels, as well as the interaction of species and P levels and of Species and N_Si levels (Model 1: Species*N_Si levels + Species*P levels) on both growth and DA production were tested.

In the second model, using end-point fluorescence as a proxy for growth, and quantifying DA per unit of fluorescence (n=243, 3 replicate per strain and per medium): the approach was similar, but considering the Strains as random effect nested in Species (Model 2: Species*N_Si levels + Species*P levels + error(strain)).

Finally, a third model was used to test statistical differences within each species, using only end-point fluorescence units as a proxy for growth, and quantifying DA per unit of fluorescence (n=81 for each species). Similar to Model 1, but considering strains rather than species as the explanatory variable (Model 3: Strains* N_Si levels + Strains*P levels).

For all models, normality of data was checked and when necessary data were log10 transformed or power transformed using the "car" package and the "powertransform" function (R version 3.2.4)

3. RESULTS

3.1. Nutrients that influence growth

The relationships between cell counts and chlorophyll fluorescence for each species were strong (Supplementary Fig. 2).

For all three species, growth in terms of final cell concentrations and chlorophyll fluorescence was highest when all nutrients were highest (i.e. for media M5, M6, M8 and M9). In contrast, lowest cell densities were found in low phosphate and low N_Si (i.e. N and Si have equivalent initial concentrations; see Methods) media (for media M1, M2, M3, M4, M7) (Fig. 2; Supplementary Fig.3).

Phosphate levels (P levels) had the strongest effect on cell densities (37% of the variance explained in terms of cell count, Table 2), especially because cell densities were extremely limited by low phosphate concentrations (media M1, M2 and M3) for all species (Fig. 2). The influence of low initial N_Si levels on cell densities was less dramatic (14% of the variance explained; Table 2), especially for *P. australis* and *P. fraudulenta*. These two species had their lowest cell densities in low-phosphate media (M2 and M3; 10.7 and 12.0 cells.μl^{-1} for *P. australis*; 25.2 and 33.2 cells.μl^{-1} for *P. fraudulenta*, respectively) in comparison to low N_Si media (M4 and M7; 22.8 and 24.1 cells.μl^{-1} for *P. australis*; 66.9 and 50.8 cells.μl^{-1} for *P. fraudulenta*) (Fig. 2). In terms of final fluorescence (as a proxy for growth), the difference between P and N_Si initial levels was less pronounced, but phosphate initial levels also represented the strongest effect overall (P levels represented 29% of the variance; while N_Si levels represented 22% of the variance) (Supplementary Fig.3; Supplementary Table 2) .

Differences in cell densities could also be observed between species (i.e the species effect explained 10% of the variance explained; Table 2). More specifically, *P. australis* presented lower densities across all media (densities ranging from 10.7 to 42.6 cell.μl^{-1}, whilst the other species had densities up to 137.1 cells.μl^{-1}; Fig. 2). Similar trends were observed using end-point fluorescence as a proxy for growth (Supplementary Fig. 3).

The interaction of cell densities and different nutrient levels (P and/or N_Si) among the three species were not significant (Table 2). However, we may note that cell densities were rather similar in media M5, M6, M8 and M9 for *P. australis* and *P. fraudulenta*, whilst *P. pungens* displayed higher cell densities in M6 and M9 than in M5 and M8 (Fig.2). Similar patterns were observed for fluorescence (Supplementary Fig. 3), and interaction effects (i.e fluorescence and nutrient levels) were only moderate (significant interactions explained 11% of the variance) (Supplementary Table 2).

Considering intra-species variation (i.e. fluorescence used as a proxy for growth), nutrients once again appeared to be the strongest factor contributing to differences in cell densities within all three species (P and N_Si combined represented 37, 66, and 75% of the variance for *P. australis*, *P. fraudulenta* and *P. pungens*, respectively; Supplementary Table 3), and in general strains grew more in mediums with high nutrient concentrations (i.e. media M5, M6, M8, M9) (Supplementary Fig. 4). There were some moderate differences amongst conspecific strains (8, 10 and 2% of the variance explained in *P. australis*, *P. fraudulenta* and *P. pungens*, respectively; Supplementary Table 3). However, within *P. australis*, one strain (Pa1) was significantly different from its conspecifics, as it displayed higher cell densities in media M1 and M2, and its lowest densities were found in M6 (40% of the variance was explained by a strong interaction Strain*P levels and Strain*N_Si levels together; Supplementary Table 3; Supplementary Fig. 4). For *P. fraudulenta* and *P. pungens*, the Strain*Media interactions were only moderate (11% and 4% of variance explained for *P. fraudulenta* and *P. pungens*, respectively; Supplementary Table 3)..

3.2. Particulate and dissolved DA

Trends in total domoic acid production (i.e. TDA = particulate DA (PDA) + dissolved DA (DDA)) revealed that inter-species variation was important (59% and 52% of the variance explained using DA.cell^{-1} and DA.fluorescence $^{-1}$, respectively; Table 2, Supplementary Table 2) and *P. australis* had the highest DA concentrations, with up to 0.47 pg.cell^{-1} (+/- 0.33 SE) (Fig. 3A).

When looking at DDA and PDA independently, *P. pungens* and *P. fraudulenta* DDA values fell below the detection limit, whilst *P. australis* had its highest DA values in the DDA category (Fig. 3A and B,). Indeed, DDA contributed to more than 76% of the total DA concentrations in *P. australis* (Fig. 3A). In contrast, PDA was observed in all three species. Inter-species differences were also observed in the PDA category, and the highest DA concentrations were once more found for *P. australis* (Table 2; 59% and 25% of the variance explained for TDA and PDA, respectively). Matching patterns were found when looking at DA per unit of fluorescence (Supplementary Fig. 5A).

Intra-species variations were found between different *P. australis* strains for both TDA and PDA (Supplementary Table 3; 59% and 25% of the variance explained for TDA and PDA, respectively). Strain Pa1 reached the highest values overall for both TDA and PDA, while Pa3 had the lowest values of all three *P. australis* strains (Supplementary Fig. 6). Within *P. fraudulenta* and *P. pungens*, DA production was only marginally attributed to inter-strain differences (3% and 2% of the variance explained; Supplementary Table 3) and primarily attributed to P levels (50% and 31% of the variance explained for *P. fraudulenta* and *P. pungens*, respectively; Supplementary Table 3).

3.3 Low initial phosphate induced DA

When looking at differences in DA production in relation to different nutrient ratios and concentrations, P levels was the second factor (after species) affecting TDA and the strongest factor for PDA (Table 2; 14% and 27% of the variance explained for TDA and PDA, respectively). Opposite to the trends observed with cell densities (i.e. high cell densities when initial N_Si and P concentrations were low), DA was highest in media with low initial N_Si and P concentrations, and particularly when initial phosphate was low (Fig. 3B). Indeed, for all DA categories (particulate, dissolved and total) and across all species, DA was highest in the low-phosphate media M1, M2 and M3 (Fig. 3). *P. australis* reached its highest total DA (TDA) in the low-phosphate media (M1, M2 and M3), with 0.33 pg.cell^{-1}, 0.35 pg.cell^{-1} and 0.47 pg.cell^{-1}, respectively. Total DA levels of *P. fraudulenta* and *P. pungens* (equivalent to PDA since there was no DDA for these species) were also highest in low-phosphate media, (Fig. 3B). *P. fraudulenta* reached a maximum PDA in medium M1 (0.05 pg.cell^{-1}) followed by M2 and M3 (0.02 and 0.01 respectively) (Fig. 3B). *P. pungens* also had the highest PDA concentrations in these three media (0.02, 0.02, and 0.01 pg.cell^{-1}). Similar trends were observed when looking at DA in relation to units of fluorescence (Supplementary Fig. 5B). Overall, diversity of nutrient conditions in terms of ratios and concentrations examined in this study revealed that, even at similar ratios (i.e N_Si: P ratios), a decrease in the concentration of phosphate (e.g media M2 and M6: ratios of 53.3 and 48, concentrations of 3 μM to 10 μM, respectively; Fig. 1) induced divergent responses: lower growth and much higher DA concentrations.

Finally, the effect of the interactions between the genotypes (species or strains) and environmental (N_Si levels or P levels) factors on DA production was only moderate (Table 2 and Supplementary Table 2 and 3; only a few percent of the variance explained). Nevertheless, the production of DA of the three species seems to

follow slightly different trends in the various media. Indeed, for a given P-level, the DA production of *P. fraudulenta* and *P. pungens* tends to be higher in the media with low N_Si levels, while it is not the case for *P. australis*. This is visible when investigating species specific differences (Fig.3 and Supplementary Fig. 5), but also when considering the intraspecific level (N_Si levels explaining 10 and 19 % of the variance in TDA production within *P. fraudulenta* and *P. pungens*, but only 1% within *P. australis*, see Supplementary Table 3). This suggests that the conditions favoring DA production are mainly preserved across the studied species but that slight species specific differences may exist.

4. DISCUSSION

This study investigated the effect of genotypes (species and strains) and environmental factors on growth (i.e in terms of cell densities at stationary phase) and domoic acid (DA) production in *Pseudo-nitzschia*. Results indicated that growth and DA production are both affected by the environmental factors examined and predominantly by low phosphate concentrations, which resulted in lower cell densities and highest DA concentrations across all species and the majority of strains studied. Growth differences between species were only moderate, but there were extreme differences in toxin concentrations, and *P. australis* appeared to be the species with the highest DA concentrations amongst the studied species. Considering strains, within *P. fraudulenta* and *P. pungens*, cell densities and DA production were mainly affected by nutrient levels, and very weakly by differences amongst strains. However, within *P. australis*, one strain displayed very different patterns in terms of both growth (cell concentrations less variable across the environments than the other

350 strains) and DA production (higher toxin production than conspecific strains and
351 highest toxin production overall).
352
353 ***4.1. Phosphate limitation an important factor for growth and DA production***
354 Many studies looking at domoic acid (DA) production in *Pseudo-nitzschia*
355 species have concentrated on the effect of varying nitrogen or silicate sources and
356 limitations. Fewer have looked at the effect of phosphate and at the effect of variable
357 Si:N:P ratios and concentrations on DA production in *Pseudo-nitzschia* (Pan et al.,
358 1996a; Fehling et al., 2004; Hagström et al., 2010; Terseleer et al., 2013).
359 Nevertheless, previous studies found that, in some *Pseudo-nitzschia* species, DA
360 production was increased under P limitation (Pan et al., 1996; Bates et al., 1998;
361 Fehling 2004). Similarly to our results, Hagström et al (2010) found that phosphate
362 deficiencies (in terms of a high N:P ratio, 290, and low phosphate concentration : 580
363 µM $NaNO_3$ to 2 µM K_2HPO_4) on two distant *Pseudo-nitzschia multiseries* strains (one
364 from California and one from Brazil) resulted in several fold increase in DA
365 production for both strains (Hagström et al., 2010), and, that DA was still produced
366 under N deficiency, but at much lower concentrations (atomic ratio N:P of 1,
367 concentrations of 36 µM $NaNO_3$, 36 µM K_2HPO_4;) (Hagström et al., 2010).
368 Importantly, the additional nutrient conditions examined in the present study
369 (different ratios and concentrations) revealed that a low concentration of phosphate
370 induced high DA concentrations and lowest cell densities regardless of the initial
371 nutrient ratio. In other words, initial P concentration and not initial ratio affected DA
372 concentration.
373 In an attempt to understand the physiological mechanism by which
374 phytoplankton toxins are produced, recent studies suggested that since secondary

375 metabolites share common precursors with growth, when growth is limited and
376 photosynthesis continues, precursors are in excess and accumulate in secondary pools,
377 where they become secondary metabolites such as DA toxins (Bates et al., 1991;
378 Terseleer et al., 2013; Van de Waal et al., 2014; Pinna et al., 2015). With that in mind,
379 Terseleer et al. (2013) and Bates et al. (1991) suggested the same mechanistic model
380 for DA whereby: provided nitrogen (N) and light are sufficient (i.e. in this instance
381 carbon and nitrogen are accumulating in secondary pools), a limitation of Si or P
382 favors DA production. The results of our study highlight that when N and Si are set to
383 an initial equivalent availability, a reduction in phosphate seems to have a stronger
384 effect on DA production than a combined N and Si reduction. Previous studies
385 showed that N and Si are independently affecting DA production (Bates et al. 1991).
386 Ideally, one would have separately varied N, Si and P ratio and concentrations to
387 untangle the respective effect of these three elements. However, in our study, it was
388 technically impossible to manage in conjunction with the nine strains investigated.
389
390
391 ***4.2. Inter and Intra species differences***
392 Even though our study highlights that a limitation in phosphate favored DA
393 production, inter and intra species differences were observed. In the present study, we
394 found that *P. australis* produced the highest DA concentrations amongst the species
395 studied. Particularly because this species released high amounts of DDA (i.e which
396 represented the highest DA category) while the others did not (or did, but at
397 undetectable values). *P. australis* is one of several ubiquitous species that are found
398 produce DA in many places of the world, such as Europe (e.g. Cusack et al., 2002;
399 Nezán et al., 2006, 2010; Klein et al., 2010; Thorel et al., 2014), North America (e.g.

400 Anderson et al., 2006; Hernández-Becerril et al., 2007; Trainer et al., 2012), New
401 Zealand (Hay et al., 2000) and South America (Sar et al., 1998). And this species has
402 been recurrently associated with high amounts of DDA (e.g. Maldonado et al., 2002;
403 Thessen et al., 2009; Martin-Jézéquel et al., 2015). On the northern west coast of
404 France, *P. australis* is responsible for the majority of toxic blooms in this area (Nézan
405 et al., 2006, 2010; Thorel et al., 2014). If the release of DDA is a physiological trait
406 and not only the result of cell mortality (which was not the case in our study) genetic
407 research in this domain could provide information for the mechanism used in the
408 release of this toxin.
409 In the present study, all the strains of *P. pungens* and *P. fraudulenta* studied
410 produced low quantities of DA. Both species are already considered as potentially
411 toxic but previous studies revealed an important variability in DA production within
412 these two species. Although *P. fraudulenta* has sometimes been found non-toxigenic
413 (Hasle 2002; Thessen et al. 2009), it has also been found capable of DA production
414 (Rhodes et al. 2013, up to 0.03 pg.cell^{-1}; Thessen et al. 2009, up to 0.667 fg.cell^{-1}),
415 with cellular DA contents often close to detection limits. *P. pungens* is a species
416 characterized by an important genetic and physiological intraspecific diversity
417 (Casteleyn et al. 2008; Hubbard et al. 2008; Lim et al. 2014; Kim et al. 2015).
418 Previous studies report that some strains do not produce DA (Bates et al 1998 and
419 references therein), while some other strains can be toxigenic (Bates et al. 1998 < 0.1
420 pg.cell^{-1}, Lelong et al. 2012 and references therein). For these two species, the DA
421 cellular concentrations measured in the present study appear in accordance with
422 previously reported cellular DA contents (Bates et al. 1998; Rhodes et al. 2013;
423 Thessen et al. 2009).
424

425 Intra-species variability has been previously observed to be stronger than inter-
426 species variability under different nitrogen sources (i.e. NO_3^-, NH_4^+ and urea; Thessen
427 et al., 2009). In our study, intra-species differences were in general only moderate.
428 However, one *P. australis* strain, Pa1, appeared to be different in terms of growth and
429 DA production. Although Pa1 had the highest DA concentrations in P-limited
430 conditions, similar cell densities and high DA concentrations were found across all
431 media. It is important to highlight that this strain was collected only one month before
432 the experiments began while the others had been in culture for at least one year.
433 Previous studies have highlighted that with time, cellular DA concentrations decrease
434 in culture (reviewed in Lelong et al., 2012). In addition to the age of the culture, some
435 studies suggest that DA production of *Pseudo-nitzschia* species may be influenced by
436 cell size (Amato et al., 2010; Lelong et al., 2012; Lundholm et al., 2012). As a result
437 of their peculiar division mode, the average diatom cell size decreases in populations
438 during vegetative multiplication, to reach a cell size threshold at which restoration of
439 large cells, via sexual reproduction, is necessary for the survival of the population
440 (Round et al., 1990; Chepurnov et al., 2004). During the present study, the *P.*
441 *australis* strain (Pa1) that produced the highest DA concentrations was characterized
442 by slightly larger cells compared to its conspecific strains (56.4 ± 1.3 µm, 47.4 ± 1.7
443 µm and 48.0 ± 2.5 µm, for Pa1, Pa2 and Pa3, respectively). However, similar
444 differences in cell sizes did not seem to affect DA production among strains of *P.*
445 *pungens* (Pp1, 51.54 ± 1.29 and Pp2, 46.72 ± 1.13; no measure available for Pp3), and
446 the two strains of *P.fraudulenta* measured had similar lengths (Pf1, 32.63 ± 1.02 µm;
447 Pf3, 34.09 ± 0.85 µm). Such results highlight that the cell size decrease alone is not
448 sufficient to explain differences in DA production, and that more profound

449 physiological modifications linked to the culture under laboratory conditions may be
450 at play.
451 Finally, as mentioned previously, a strong environmental effect on both
452 growth and DA production was identified. Some conditions favored growth (high
453 nutrient supply), and some conditions favored DA production (low phosphate). The
454 inter and intra species difference were mainly observed for DA production, at both the
455 species level, with *P. australis* producing more DA, and the strain level, with Pa1
456 producing more DA that the other two *P. australis* strains. No major genotype and
457 environment interaction was detected, meaning that the media in which the various
458 genotypes (species or strains) produced more DA and attained higher cell densities
459 were more or less the same. Such results illustrate that the various genotypes
460 investigated did not distinctively diverge in their ability to respond (a least in term of
461 DA production and cell densities) to contrasting nutrient supply. However, we may
462 note a slight impact of nitrate and silicate supplies on DA production for *P.*
463 *fraudulenta* and *P. pungens*, not found in *P. australis*. The only major exception was
464 strain Pa1, which presented a higher cell density in two of the media with a low
465 phosphate supply. But as discussed above regarding DA production, we may wonder
466 if the specificity of this strain has a genotypic, or rather a physiological basis.
467
468
469 ***4.3 Conclusions***
470 The results of the present study show important inter species differences on
471 DA production: *P. australis* being the most toxigenic. However, the mechanism of
472 DA production control by nutrient conditions was rather similar amongst the three
473 species studied with DA production stimulated by phosphorus deficiency. In addition,

474 this study demonstrates only moderate intra-specific variability, with the exception of
475 a recently isolated *P. australis* strain, advocating for a deeper understanding of the
476 influence of culture duration and life cycle on the physiology and DA production of
477 *Pseudo-nitzschia* species.
478
479
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481 ☒
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487
488 **The authors declare no conflict of interest**
489
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663
664

665

666 **FIGURE LEGENDS**

667

668 Figure 1. Matrix diagram indicating the nutrient concentrations (i.e. phosphate,
669 nitrate, silicate) for each of the nine media (M1 to M9) used in this study. In
670 parenthesis is the nutrient ratio N_Si:P is presented (nb: M1, M5 and M9 have a N:P
671 and Si:P ratio of 16:1, and "N_Si" indicate that N and Si have equivalent initial
672 concentrations). The nitrogen, silicate and phosphate levels, in relation to their
673 respective concentrations, are indicated as: low, medium and high.

674

675 Figure 2. Growth in terms of cell abundance (cells.μl^{-1}) at stationary phase for the
676 three species of *Pseudo-nitzschia* studied (all strains combined). Heat matrices
677 correspond to the nine different media, with different nutrient concentrations: P and
678 N_Si levels as in Figure 1; different grey shades represent low to high number of
679 cells. Average number of cells and standard errors are indicated for each medium.

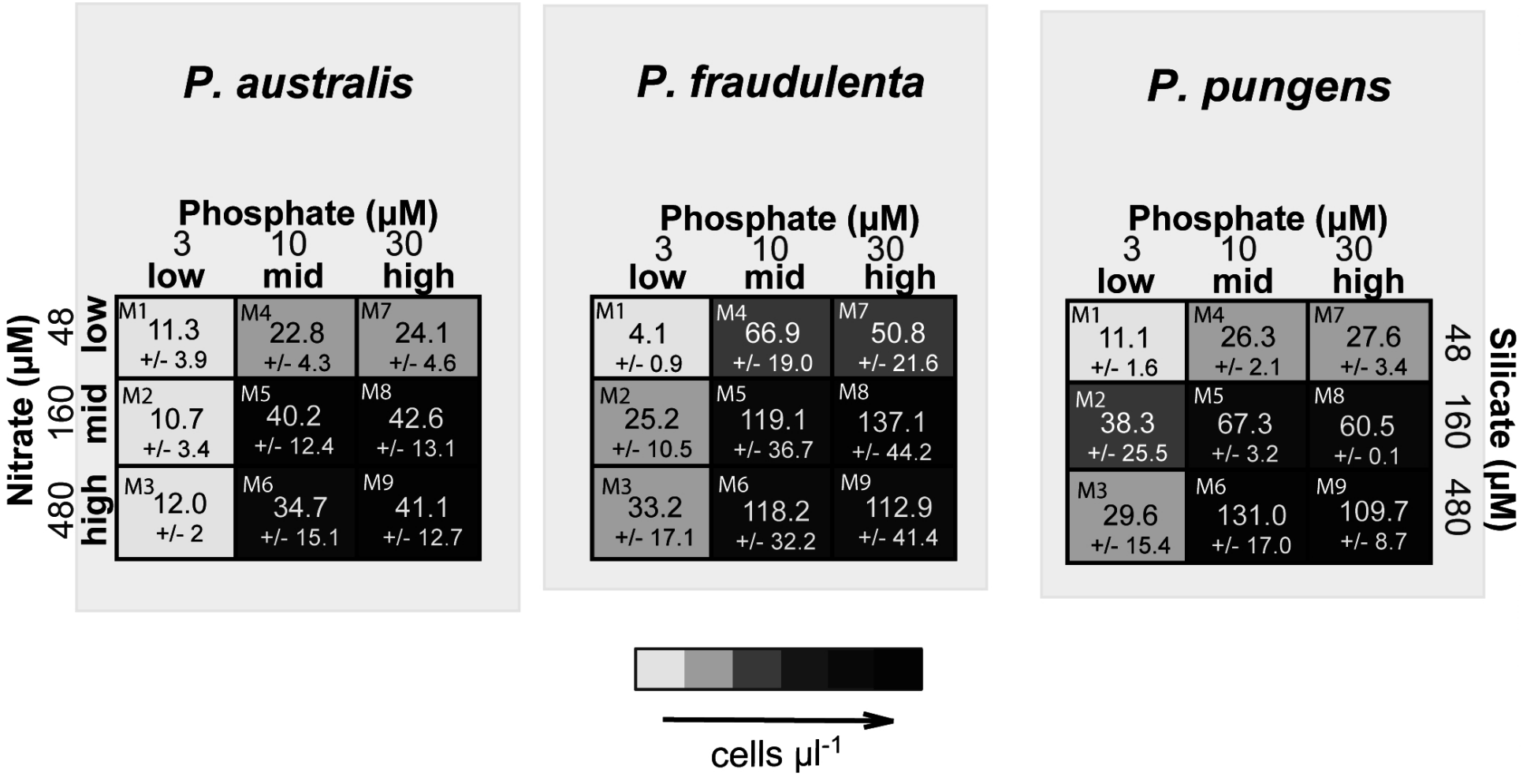
680

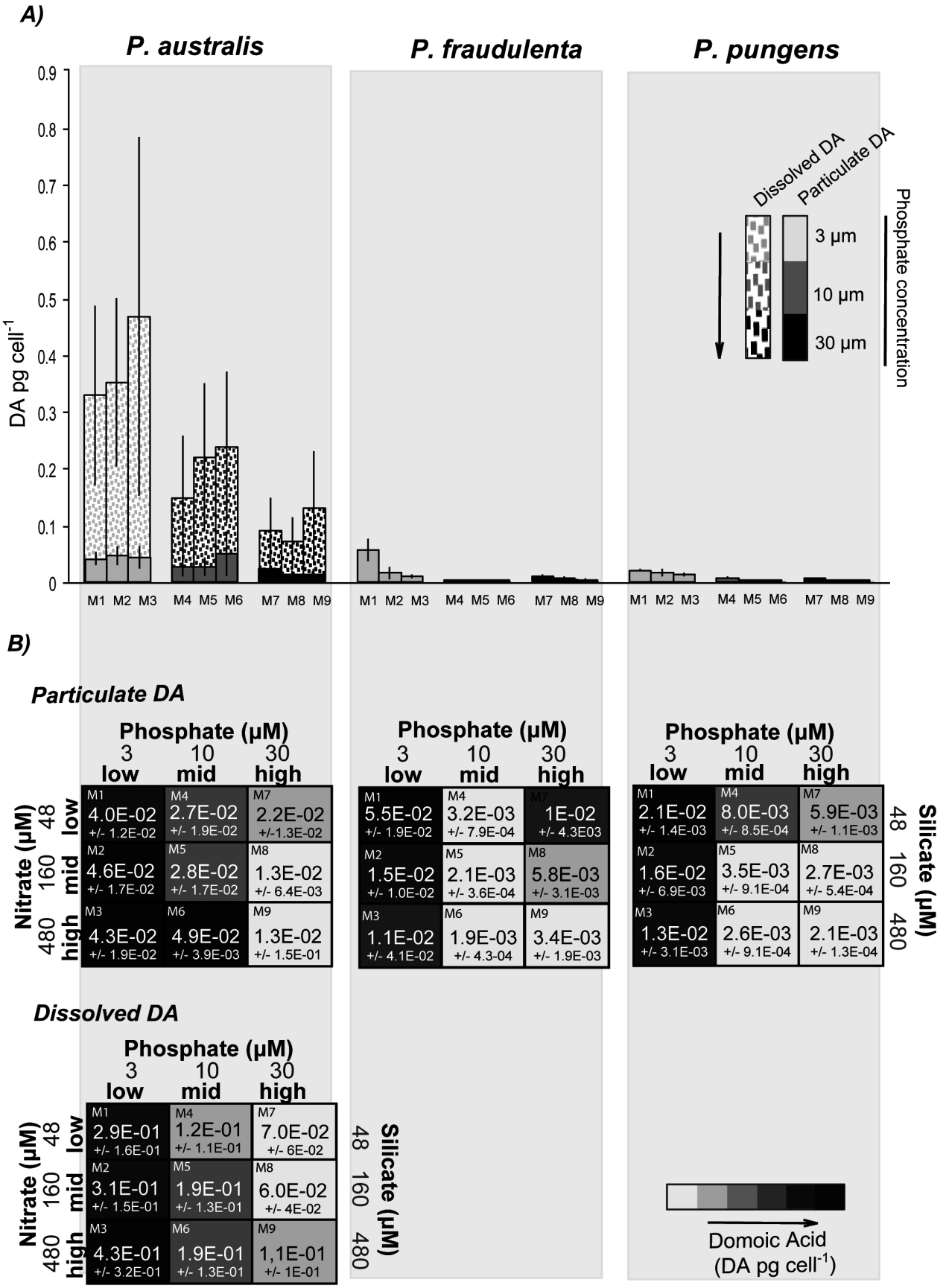
681 Figure 3. Domoic acid (DA) concentrations (pg.$cell^{-1}$) at stationary phase for the three
682 species of *Pseudo-nitzschia* studied (all strains combined) growing in different media.
683 A) DA concentrations of the different species assigned to different phosphate
684 concentrations (low, medium and high), corresponding to different shades of grey. B)
685 Heat matrices, where different grey shades represent low to high concentrations of
686 DA. Average DA and standard errors are indicated for each medium.

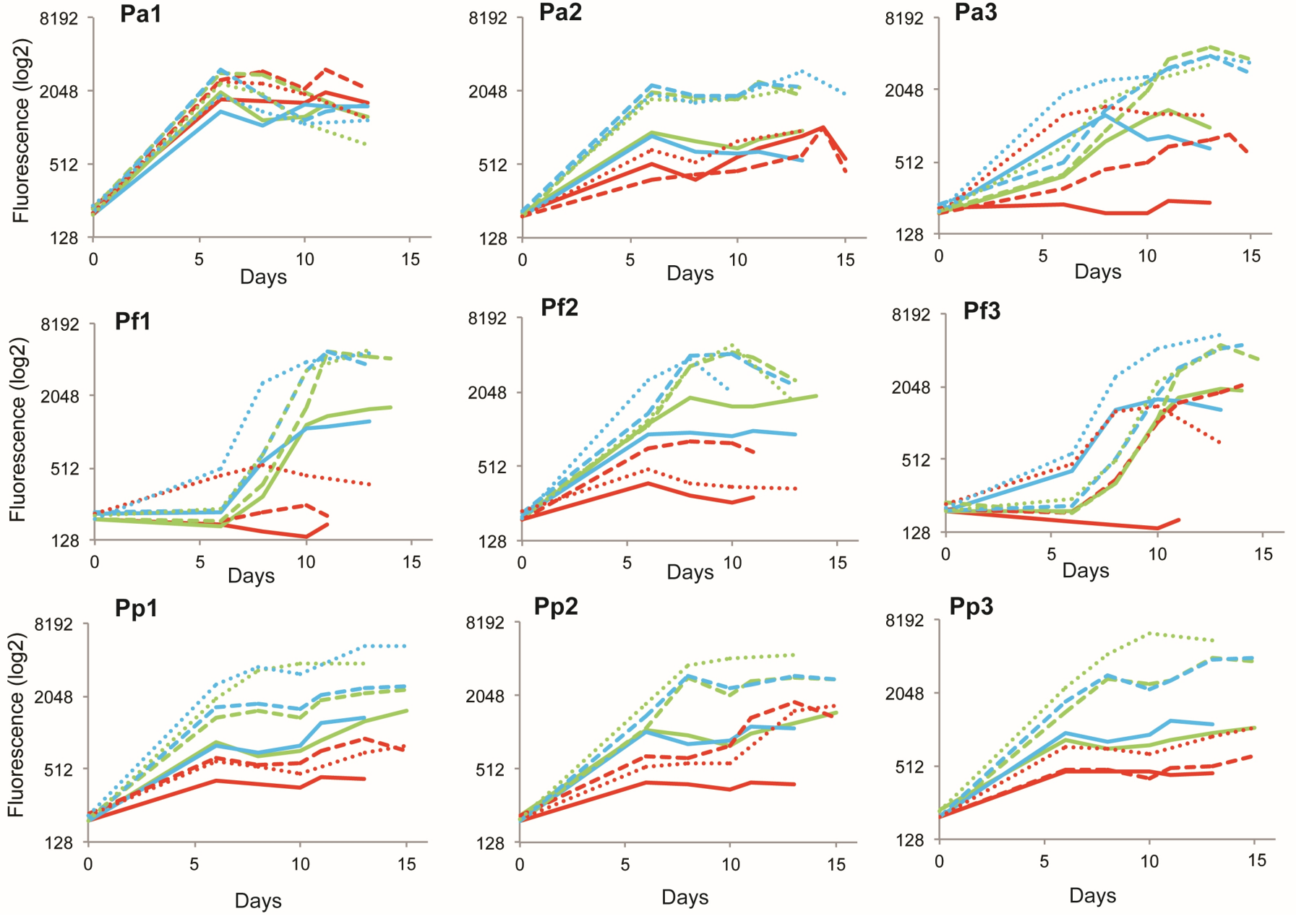
687
688

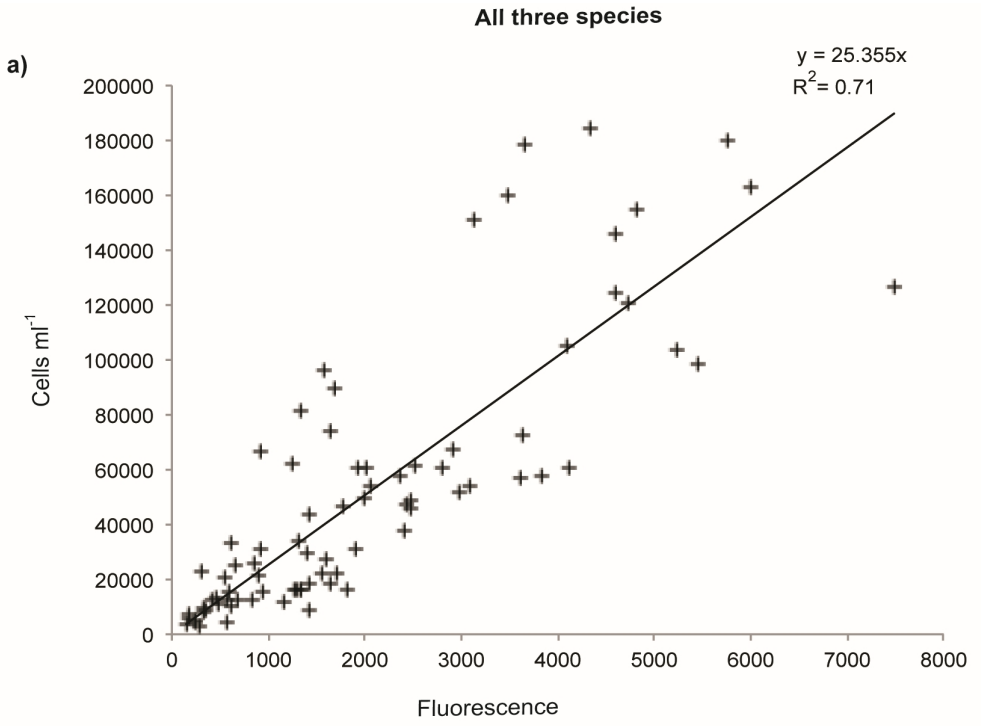
Phosphate (μM)

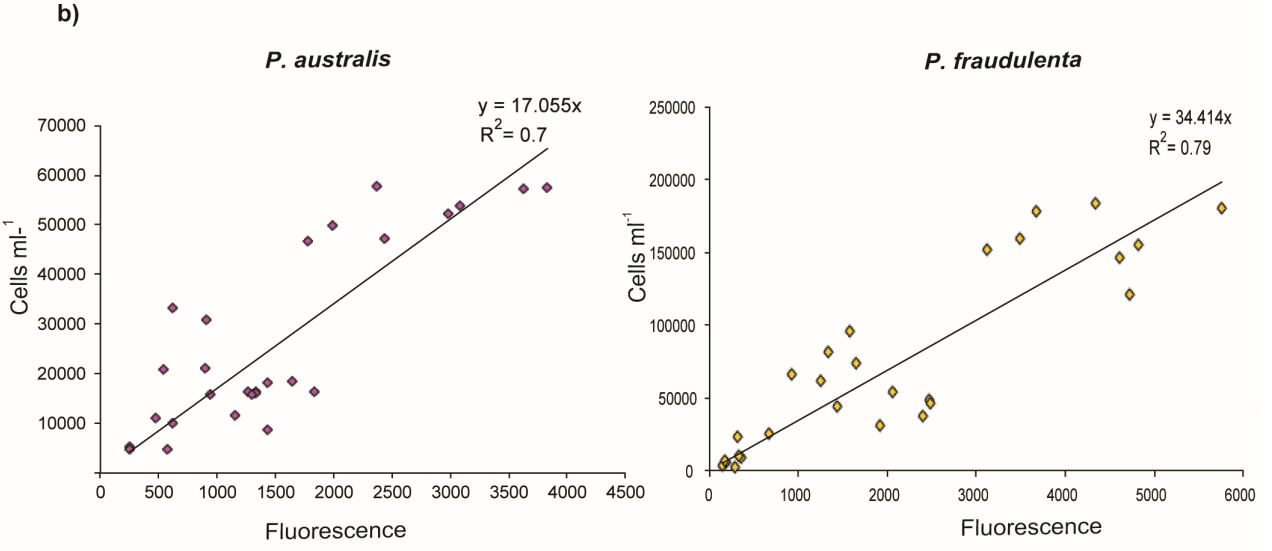
Nitrate (μM)	(low) 3	(mid) 10	(high) 30	Silicate (μM)
(low) 48	M1 **(16)**	M4 (4.8)	M7 (1.6)	48
(mid) 160	M2 (53.3)	M5 **(16)**	M8 (5.3)	160
(high) 480	M3 (160)	M6 (48)	M9 **(16)**	480

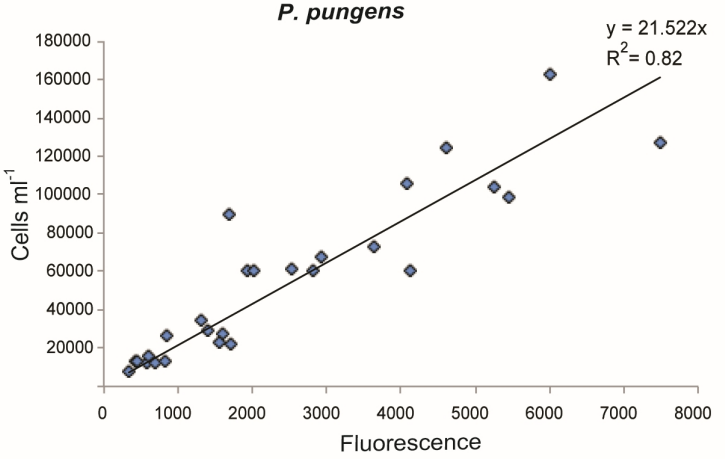

P. australis
Phosphate (µM)
3 low
10 mid
30 high
Nitrate (µM)
48 low
160 mid
480 high
M1 11.3 +/- 3.9
M4 22.8 +/- 4.3
M7 24.1 +/- 4.6
M2 10.7 +/- 3.4
M5 40.2 +/- 12.4
M8 42.6 +/- 13.1
M3 12.0 +/- 2
M6 34.7 +/- 15.1
M9 41.1 +/- 12.7
P. fraudulenta
Phosphate (µM)
3 low
10 mid
30 high
M1 4.1 +/- 0.9
M4 66.9 +/- 19.0
M7 50.8 +/- 21.6
M2 25.2 +/- 10.5
M5 119.1 +/- 36.7
M8 137.1 +/- 44.2
M3 33.2 +/- 17.1
M6 118.2 +/- 32.2
M9 112.9 +/- 41.4
P. pungens
Phosphate (µM)
3 low
10 mid
30 high
M1 11.1 +/- 1.6
M4 26.3 +/- 2.1
M7 27.6 +/- 3.4
M2 38.3 +/- 25.5
M5 67.3 +/- 3.2
M8 60.5 +/- 0.1
M3 29.6 +/- 15.4
M6 131.0 +/- 17.0
M9 109.7 +/- 8.7
Silicate (µM)
48 160 480
cells µl-1

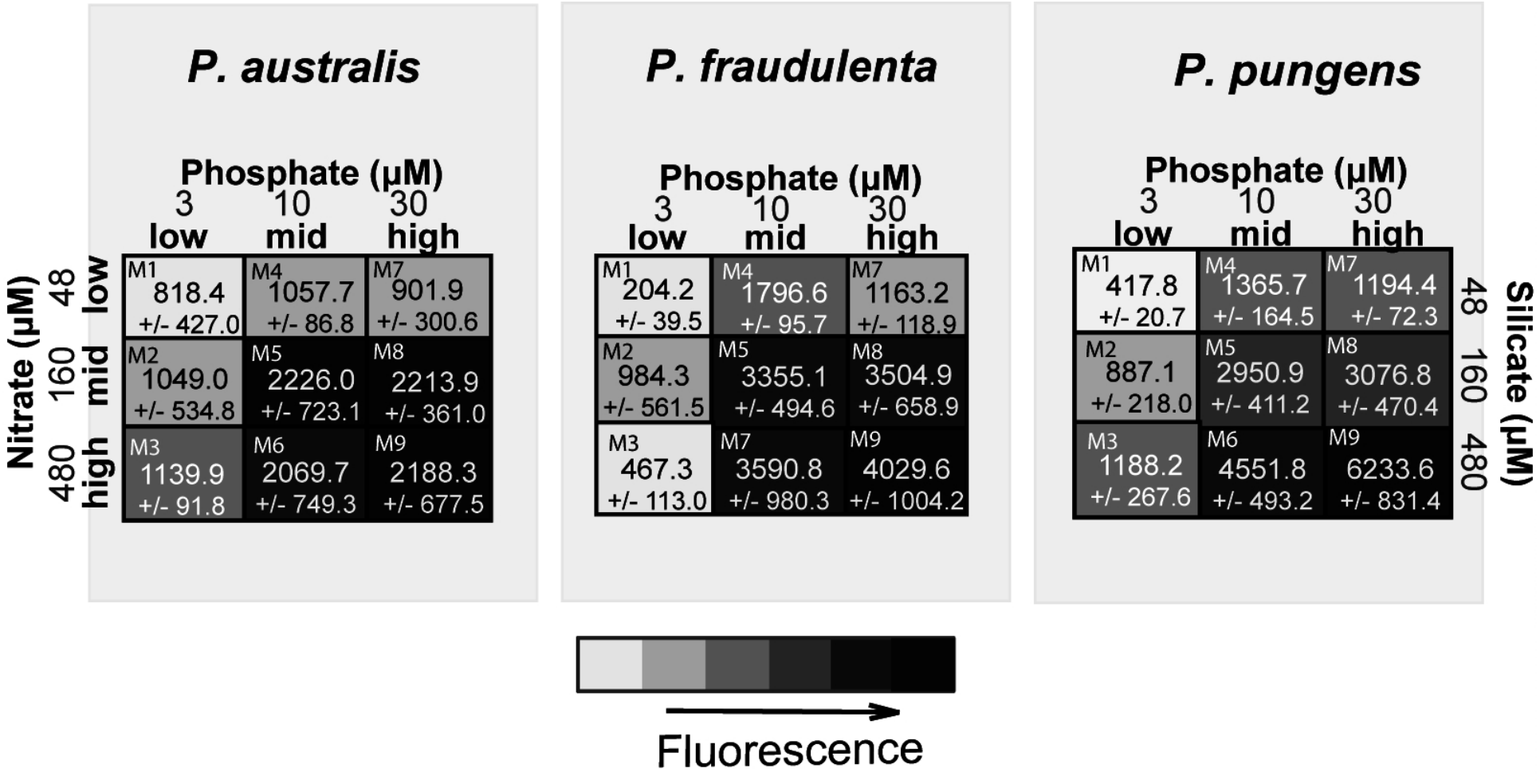

A)
P. australis
P. fraudulenta
P. pungens
DA pg cell-1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
M1 M2 M3 M4 M5 M6 M7 M8 M9
Dissolved DA
Particulate DA
3 µm
10 µm
30 µm
Phosphate concentration
B)
Particulate DA
Phosphate (µM)
3 low
10 mid
30 high
Nitrate (µM)
48 low
160 mid
480 high
Silicate (µM)
M1 4.0E-02 +/- 1.2E-02
M4 2.7E-02 +/- 1.9E-02
M7 2.2E-02 +/-1.3E-02
M2 4.6E-02 +/- 1.7E-02
M5 2.8E-02 +/- 1.7E-02
M8 1.3E-02 +/- 6.4E-03
M3 4.3E-02 +/- 1.9E-02
M6 4.9E-02 +/- 3.9E-03
M9 1.3E-02 +/- 1.5E-01
M1 5.5E-02 +/- 1.9E-02
M4 3.2E-03 +/- 7.9E-04
M7 1E-02 +/- 4.3E03
M2 1.5E-02 +/- 1.0E-02
M5 2.1E-03 +/- 3.6E-04
M8 5.8E-03 +/- 3.1E-03
M3 1.1E-02 +/- 4.1E-02
M6 1.9E-03 +/- 4.3-04
M9 3.4E-03 +/- 1.9E-03
M1 2.1E-02 +/- 1.4E-03
M4 8.0E-03 +/- 8.5E-04
M7 5.9E-03 +/- 1.1E-03
M2 1.6E-02 +/- 6.9E-03
M5 3.5E-03 +/- 9.1E-04
M8 2.7E-03 +/- 5.4E-04
M3 1.3E-02 +/- 3.1E-03
M6 2.6E-03 +/- 9.1E-04
M9 2.1E-03 +/- 1.3E-04
Dissolved DA
Phosphate (µM)
3 low
10 mid
30 high
Nitrate (µM)
48 low
160 mid
480 high
Silicate (µM)
M1 2.9E-01 +/- 1.6E-01
M4 1.2E-01 +/- 1.1E-01
M7 7.0E-02 +/- 6E-02
M2 3.1E-01 +/- 1.5E-01
M5 1.9E-01 +/- 1.3E-01
M8 6.0E-02 +/- 4E-02
M3 4.3E-01 +/- 3.2E-01
M6 1.9E-01 +/- 1.3E-01
M9 1,1E-01 +/- 1E-01
Domoic Acid
(DA pg cell-1)


Pa1
Pa2
Pa3
Pf1
Pf2
Pf3
Pp1
Pp2
Pp3
Fluorescence (log2)
8192
2048
512
128
0
5
10
15
Days

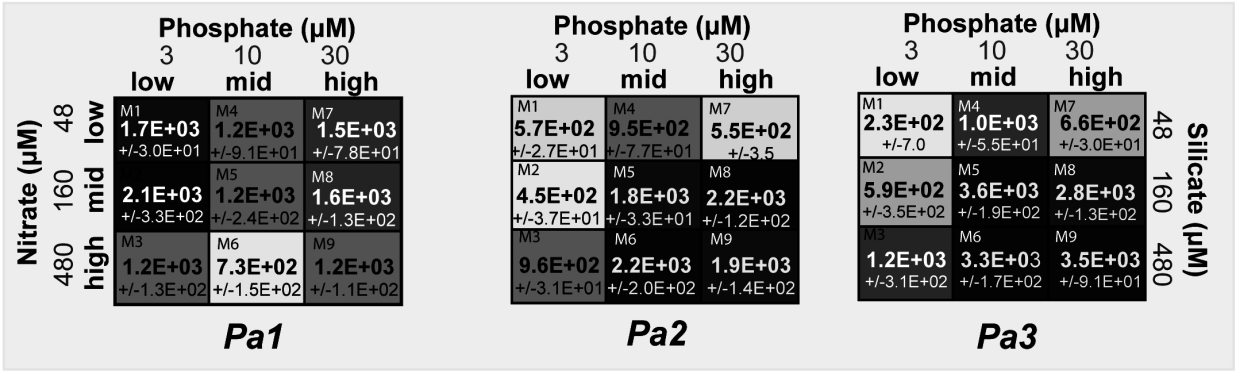

a)
All three species
y = 25.355x
R2= 0.71
Cells ml-1
Fluorescence
0
20000
40000
60000
80000
100000
120000
140000
160000
180000
200000
0
1000
2000
3000
4000
5000
6000
7000
8000


b)
P. australis
y = 17.055x
R2= 0.7
Cells ml-1
Fluorescence
10000
20000
30000
40000
50000
60000
70000
0
500
1000
1500
2000
2500
3000
3500
4000
4500
P. fraudulenta
y = 34.414x
R2= 0.79
Cells ml-1
Fluorescence
0
50000
100000
150000
200000
250000
0
1000
2000
3000
4000
5000
6000

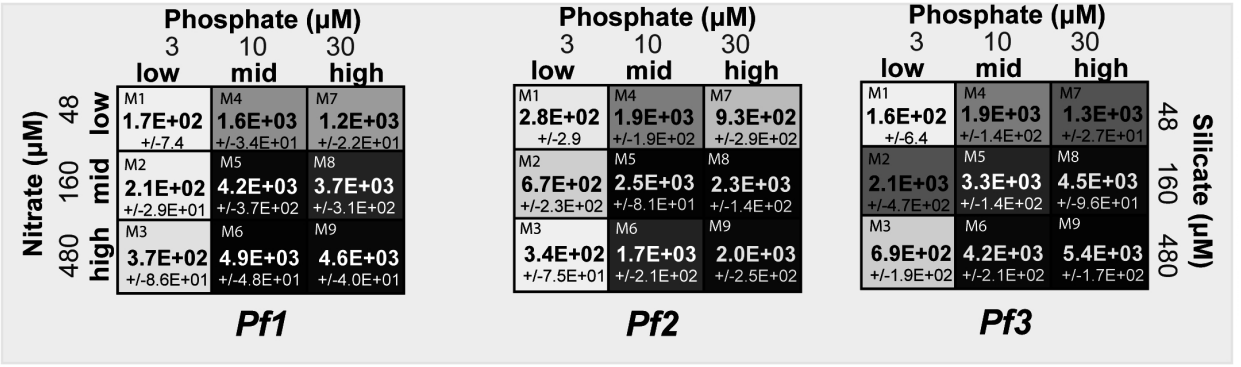

P. pungens
y = 21.522x
R2= 0.82
Cells ml-1
Fluorescence
20000
40000
60000
80000
100000
120000
140000
160000
180000
0
1000
2000
3000
4000
5000
6000
7000
8000


P. australis
Phosphate (µM)
3 low
10 mid
30 high
Nitrate (µM)
48 low
160 mid
480 high
M1 818.4 +/- 427.0
M4 1057.7 +/- 86.8
M7 901.9 +/- 300.6
M2 1049.0 +/- 534.8
M5 2226.0 +/- 723.1
M8 2213.9 +/- 361.0
M3 1139.9 +/- 91.8
M6 2069.7 +/- 749.3
M9 2188.3 +/- 677.5
P. fraudulenta
Phosphate (µM)
3 low
10 mid
30 high
M1 204.2 +/- 39.5
M4 1796.6 +/- 95.7
M7 1163.2 +/- 118.9
M2 984.3 +/- 561.5
M5 3355.1 +/- 494.6
M8 3504.9 +/- 658.9
M3 467.3 +/- 113.0
M7 3590.8 +/- 980.3
M9 4029.6 +/- 1004.2
P. pungens
Phosphate (µM)
3 low
10 mid
30 high
Silicate (µM)
48
160
480
M1 417.8 +/- 20.7
M4 1365.7 +/- 164.5
M7 1194.4 +/- 72.3
M2 887.1 +/- 218.0
M5 2950.9 +/- 411.2
M8 3076.8 +/- 470.4
M3 1188.2 +/- 267.6
M6 4551.8 +/- 493.2
M9 6233.6 +/- 831.4
Fluorescence

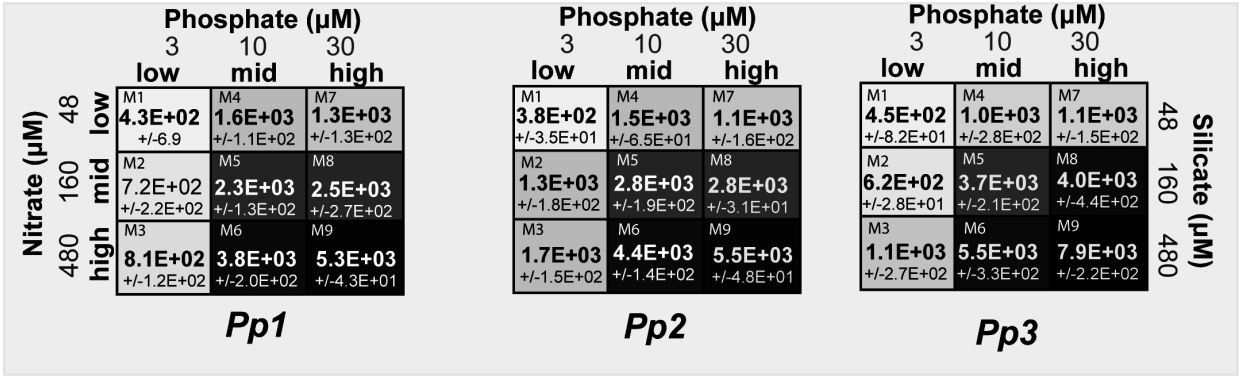
P. australis

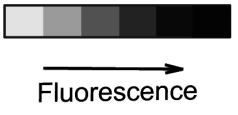


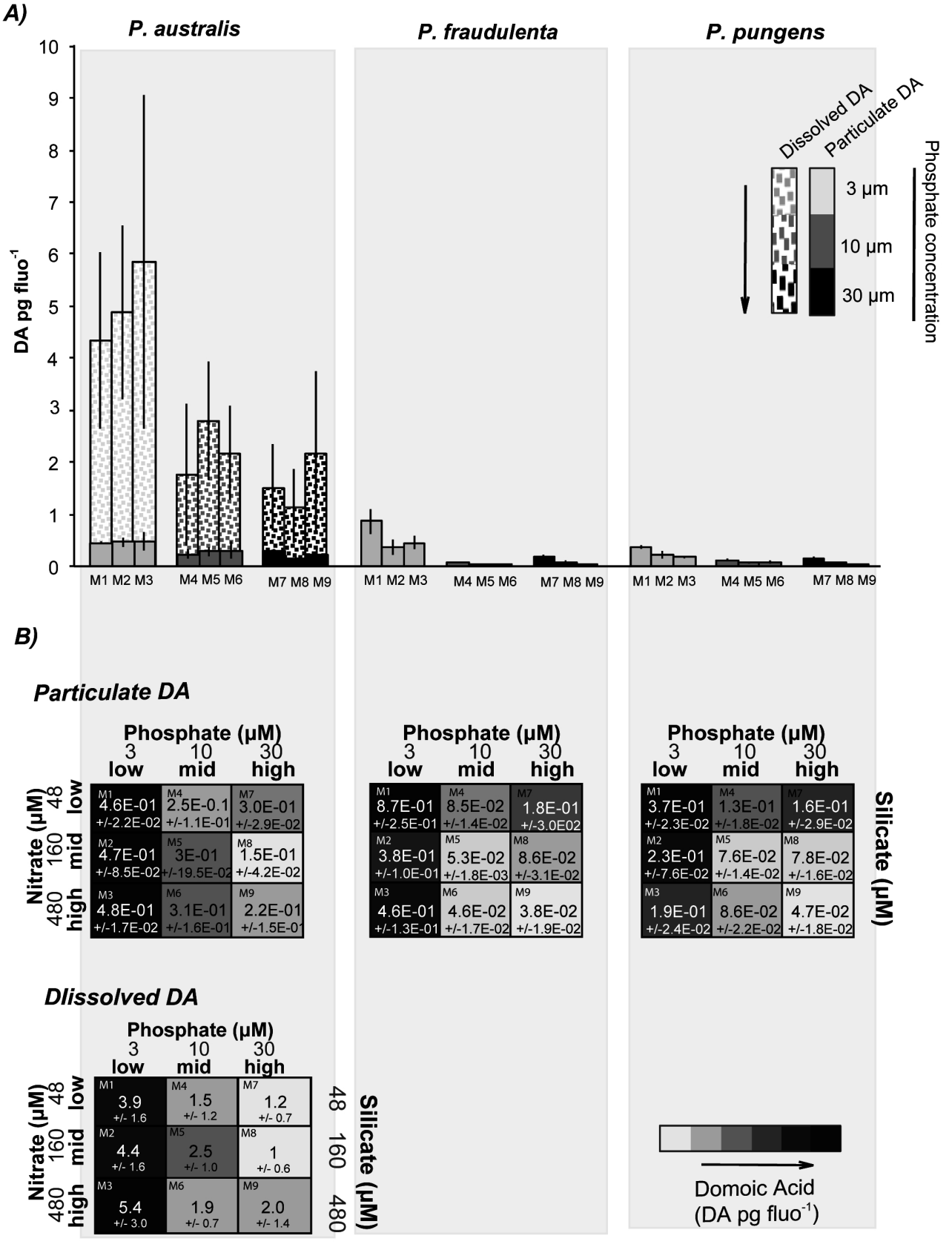
P. fraudulenta



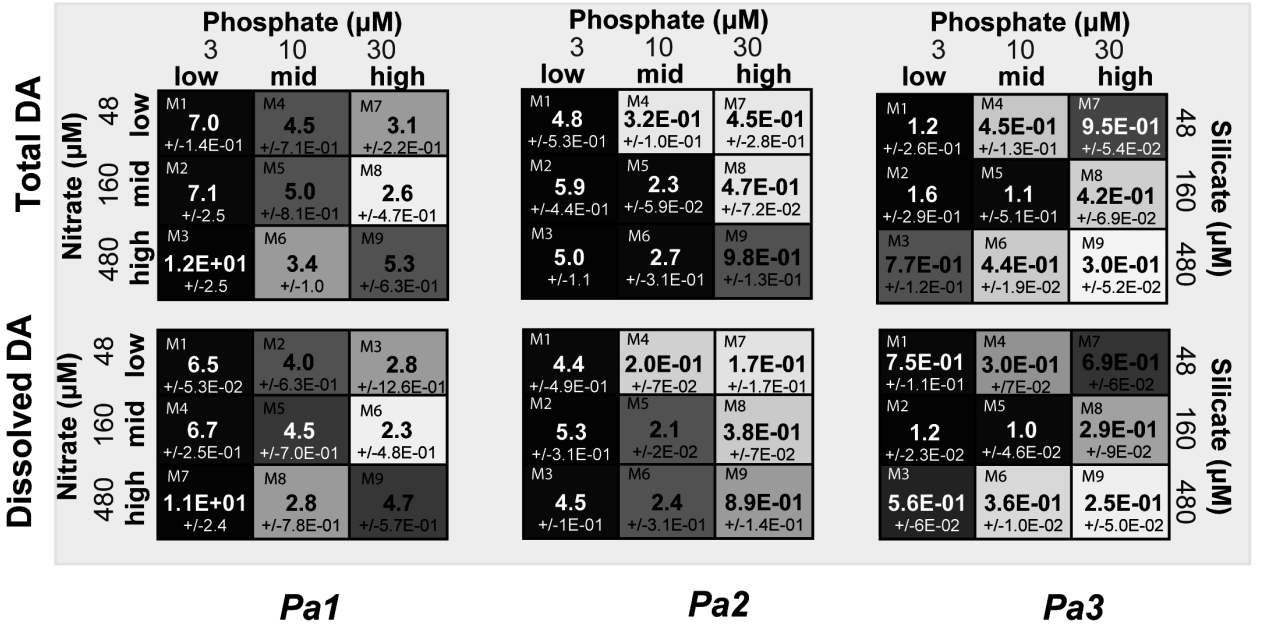
P. pungens



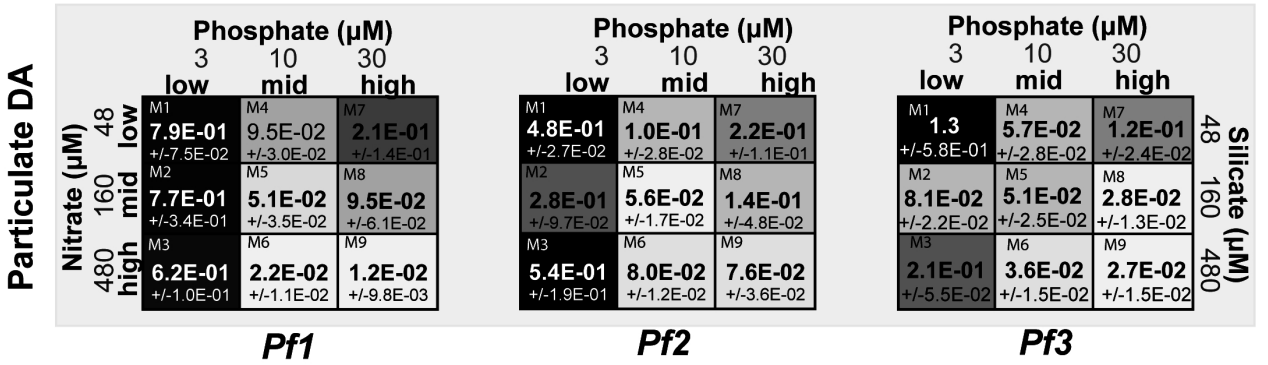



A)
P. australis
P. fraudulenta
P. pungens
DA pg fluo-1
Dissolved DA
Particulate DA
3 µm
10 µm
30 µm
Phosphate concentration
M1 M2 M3
M4 M5 M6
M7 M8 M9
B)
Particulate DA
Phosphate (µM)
3 low
10 mid
30 high
Nitrate (µM)
48 low
160 mid
480 high
M1 4.6E-01 +/-2.2E-02
M4 2.5E-0.1 +/-1.1E-01
M7 3.0E-01 +/-2.9E-02
M2 4.7E-01 +/-8.5E-02
M5 3E-01 +/-19.5E-02
M8 1.5E-01 +/-4.2E-02
M3 4.8E-01 +/-1.7E-02
M6 3.1E-01 +/-1.6E-01
M9 2.2E-01 +/-1.5E-01
M1 8.7E-01 +/-2.5E-01
M4 8.5E-02 +/-1.4E-02
M7 1.8E-01 +/-3.0E02
M2 3.8E-01 +/-1.0E-01
M5 5.3E-02 +/-1.8E-03
M8 8.6E-02 +/-3.1E-02
M3 4.6E-01 +/-1.3E-01
M6 4.6E-02 +/-1.7E-02
M9 3.8E-02 +/-1.9E-02
M1 3.7E-01 +/-2.3E-02
M4 1.3E-01 +/-1.8E-02
M7 1.6E-01 +/-2.9E-02
M2 2.3E-01 +/-7.6E-02
M5 7.6E-02 +/-1.4E-02
M8 7.8E-02 +/-1.6E-02
M3 1.9E-01 +/-2.4E-02
M6 8.6E-02 +/-2.2E-02
M9 4.7E-02 +/-1.8E-02
Silicate (µM)
Dlissolved DA
M1 3.9 +/- 1.6
M4 1.5 +/- 1.2
M7 1.2 +/- 0.7
M2 4.4 +/- 1.6
M5 2.5 +/- 1.0
M8 1 +/- 0.6
M3 5.4 +/- 3.0
M6 1.9 +/- 0.7
M9 2.0 +/- 1.4
Domoic Acid
(DA pg fluo-1)

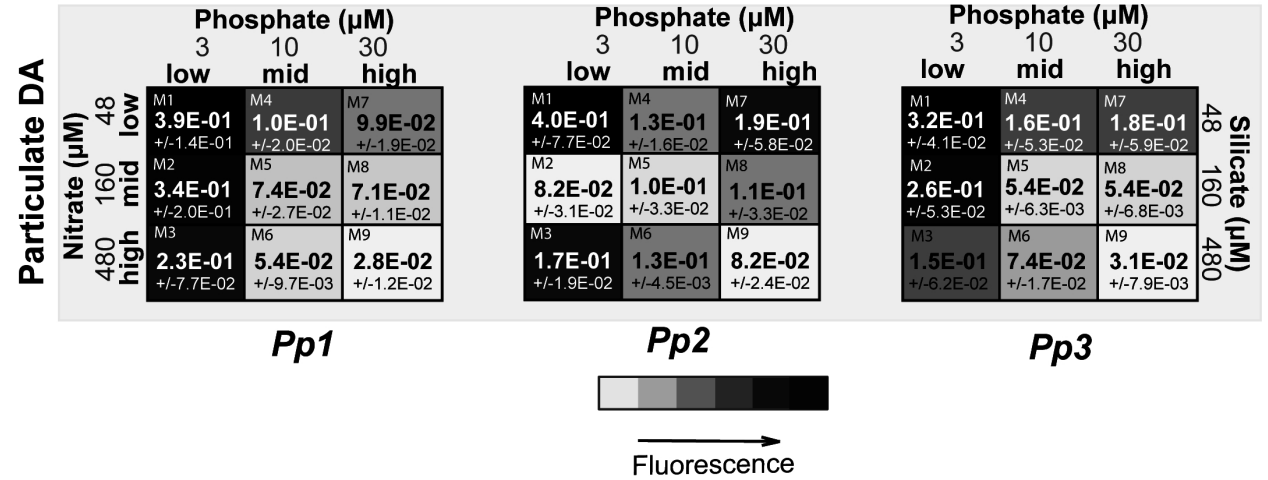
P. australis



P. fraudulenta



P. pungens



Supplementary Figure 1. Growth curves (log2 fluorescence) for each of the nine assayed strains. Each panel corresponds to a strain. Each curve indicates the mean of three replicate assays. Media with low, medium, and high initial phosphate concentrations are in red, green and blue, respectively. Media with low, medium, and high initial nitrate and silicate concentrations are in full, dashed and dotted lines, respectively

Supplementary Figure 2. Cell abundance versus fluorescence units plots.
To be able to use fluorescence as a proxy for growth we first looked at the relationship between number of cells and fluorescence at collection time (i.e at collection time: stationary phase). Overall relationship number of cells versus fluorescence at stationary phase plot a) for all species studied (r^2= 0,71). b) for each species studied separately (r^2 =0,82, r^2=0,79 and r^2=0,70 for *P.pungens* *P.fraudulenta* and *P.australis* respectively).

Supplementary Figure 3. Fluorescence at collection time (stationary phase) for the 3 species of *Pseudo-nitzschia* studied. Heat matrix correspond to the 9 different mediums, with different nutrient concentrations and P and N_Si levels as in Figure 1: where grey shades correspond to low to high fluorescence values. Average units of fluorescence as well as standard errors are indicated.

Supplementary Figure 4. Fluorescence at collection time (stationary phase), for the 9 strains of *Pseudo-nitzschia* studied and 9 medias. Heat matrix represent low to high fluorescence fallowing clear to darker shades of grey. Average units of fluorescence as well as standard errors are indicated.

Supplementary Figure 5. Domoic acid (DA) concentrations per unit of fluorescence (pg fluorescence^{-1}) at stationary phase for the 3 species of *Pseudo-nitzschia* studied (all strains combined) under different medias. A) Shows DA concentrations of the different species assigned to different phosphate concentrations (low, medium and high) corresponding to different grey shades. B) Heat matrix to show values, where grey shades represent low to high concentration of DA. Average DA and standard errors are indicated for each media.

Supplementary Figure 6. Domoic acid concentrations per unit of fluorescence at stationary phase for the 9 strains of *Pseudo-nitzschia* studied and 9 medias. Heat matrix represent low to high DA unit of fluorescence^{-1} following clear to darker shades of grey. Average DA fluorescence^{-1} as well as standard errors are indicated.

Table 1. Cultures of *Pseudo-nitzschia* spp. used in the present study (nb: the collection references of the strains have also been added to enable comparisons with other studies, and the experiment started on 13th august 2015). (A) stands for strains isolated in the Atlantic and (EC) for strains from the English Channel.

Species	Collection reference	Name present study	Collection date	Station	GPS Coordinates (approximative)
P.australis	IFR-PAU-010	Pa1	14/07/15	Ouessant (A)	48.449511; -5.108088
P. australis	P1D2	Pa2	28/03/14	Anse de Dinan, Camaret-sur-Mer (A)	48.225679; -4.563602
P. australis	P6B3	Pa3	04/04/14	Môle St Anne, Plouzané (A)	48.358573; -4.551193
P. fraudulenta	PNfra2	Pf1	24/08/11	Cabourg (EC)	49.302888; -0.103549
P. fraudulenta	PNfra29	Pf2	18/09/11	Cabourg (EC)	49.302888; -0.103549
P. fraudulenta	PNfra31	Pf3	11/07/11	COMOR 41(EC)	49.414444; -0.408889
P. pungens	PNpun47	Pp1	24/08/11	Cabourg (EC)	49.302888; -0.103549
P. pungens	PNpun66	Pp2	29/05/12	Ouistreham (EC)	49.290574; -0.246028
P. pungens	PNpun102	Pp3	21/08/12	Luc sur Mer (EC)	49.320109; -0.351193

Table 2. Statistical results. ANOVA tests for cell density and domoïc acid concentration (TDA: total domoïc acid; PDA: Particulate domoïc acid). For these analyses a log10 transformation was used. For p-values < 0.05. effect sizes (proportion of the variance explained by the factor) are reported. Significant p values are highlighted in italic bold.

Analysis	ANOVA Test : Phosphate and Nitrate_Silicate levels	Effect size	Df	Sum of Sq	F	*P*-value
Cell density log10 cells	**(Species*P levels)+(Species*N_Si levels)**					
	Species	**0.1**	2	1.725	10.989	***7.6e-05***
	P levels	**0.37**	2	6.170	39.316	***5.71e-12***
	N_Si levels	**0.14**	2	2.234	14.236	***7.24e-06***
	Species: P levels	0.03	4	0.516	1.646	0.173
	Species: N_Si levels	0.04	4	0.636	2.026	0.101
	Residuals		66	5.179		
TDA log10 pg cell^{-1}	**(Species*P levels)+(Species*N_Si levels)**					
	Species	**0.59**	2	29.770	95.349	***<2e-16***
	P levels	**0.14**	2	7.09	22.708	***3.13e-08***
	N_Si levels	0.02	2	0.922	2.953	0.059
	Species: P levels	0.02	4	0.833	1.334	*0.266*
	Species: N_Si levels	0.03	4	1.297	2.077	*0.094*
	Residuals		66	10.303		
PDA log10 pg cell^{-1}	**(Species*Plevels)+(Species*N_Silevels)**					
	Species	**0.25**	2	5.572	24.544	***1.07e-08***
	Plevels	**0.27**	2	5.992	26.395	***3.78e-09***
	N_Si levels	**0.07**	2	1.538	6.776	***0.00211***
	Species: P levels	0.05	4	0.945	2.081	0.093
	Species: N_Si levels	0.03	4	0.565	1.244	0.301
	Residuals		66	7.492		

Supplementary Table 1. Total days of growth and on stationary phase prior to collection for each strain in each of the nine mediums of this study

Species	Strain	Media	Days in stationary phase	Total days of growth
P. australis	Pa1	M1	7	13
		M2	7	13
		M3	7	13
		M4	7	13
		M5	7	13
		M6	7	13
		M7	7	13
		M8	7	13
		M9	7	13
	Pa2	M1	6	15
		M2	7	15
		M3	7	13
		M4	7	13
		M5	7	13
		M6	7	13
		M7	7	13
		M8	7	13
		M9	7	15
	Pa3	M1	7	13
		M2	5	15
		M3	7	13
		M4	5	13
		M5	5	15
		M6	5	13
		M7	5	13
		M8	5	15
		M9	7	15
P. fraudulenta	Pf1	M1	5	11
		M2	5	11
		M3	5	13
		M4	5	14
		M5	4	14
		M6	4	13
		M7	4	13
		M8	4	13
		M9	5	13
	Pf2	M1	5	11
		M2	5	11
		M3	7	13
		M4	7	14
		M5	5	13
		M6	5	13
		M7	7	13
		M8	5	13
		M9	4	10
	Pf3	M1	5	11
		M2	5	14
		M3	5	13
		M4	4	14
		M5	5	15
		M6	4	13
		M7	5	13
		M8	4	14
		M9	5	13
P. pungens	Pp1	M1	7	13
		M2	9	15
		M3	9	15
		M4	9	15
		M5	9	15
		M6	7	13
		M7	7	13
		M8	9	15
		M9	9	15
	Pp2	M1	7	13
		M2	9	15
		M3	9	15
		M4	9	15
		M5	7	15
		M6	5	13
		M7	7	13
		M8	7	15
		M9	7	15
	Pp3	M1	7	13
		M2	9	15
		M3	9	15
		M4	9	15
		M5	7	15
		M6	6	13
		M7	7	13
		M8	7	15
		M9	9	15

Supplementary Table 2. Statistical results from fluorescence data. ANOVA tests for fluorescence and domoïc acid concentration (TDA: total domoïc acid; PDA: Particulate domoïc acid). For p-values < 0.05 effect sizes (proportion of the variance explained by the factor) are reported. Significant p values are highlighted in italic bold.

Analysis	ANOVA Test: Phosphate and Nitrate_Silicate levels	Effect size	Df	Sum of Sq	F	*P*-value
Fluorescence	**(Species*P levels)+(Species*N_Si levels) +Error(Strain)**					
	Species	0.06	2	34810989	2.958	0.128
	P levels	**0.291**	2	184432785	124.89	***<2e-16***
	N_Si levels	**0.225**	2	143009670	96.84	***<2e-16***
	Species: P levels	**0.053**	4	33665195	11.40	***1.97e-08***
	Species: N_Si levels	**0.062**	4	39431540	13.35	***9.14e-10***
	Residuals		222	163915784		
Total Domoic Acid (pg fluorescence^{-1})	**(Species*P levels)+(Species*N_Si levels) +Error(Strain)***					
	Species	**0.516**	2	81.32	20.41	***0.0021***
	P levels	**0.139**	2	21.92	76.393	***<2e-16***
	N_Si levels	**0.019**	2	3	10.466	***4.53e-05***
	Species: P levels	**0.023**	4	3.61	6.284	***8.30e-05***
	Species: N_Si levels	**0.026**	4	4.04	7.032	***2.39e-05***
	Residuals		222	31.85		
Particulate Domoic Acid (pg fluorescence^{-1})	**(Species*P levels)+(Species*N_Si levels) +Error(Strain)***					
	Species	0.11	2	7.969	5.058	0.0516
	P levels	**0.253**	2	18.83	65.7	***<2e-16***
	N_Si levels	**0.064**	2	4.77	16.656	***1.82e-07***
	Species: P levels	**0.058**	4	4.3	7.501	***1.10e-05***
	Species: N_Si levels	**0.026**	4	1.94	3.393	***0.0102***
	Residuals		222	31.81		

* data transformed to log10

Supplementary Table 3. Statistical results of fluorescence within each *Pseudo-nitzschia* species (intra-species variation). ANOVA tests for fluorescence and domoïc acid concentration (TDA: total domoïc acid; PDA: Particulate domoïc acid). For p-values < 0.05 effect sizes (proportion of the variance explained by the factor) are reported. Significant p values are highlighted in italic bold.

Variable	Species	ANOVA Test: Phosphate and Nitrate_Silicate levels	Effect size	Df	Sum of Sq	F	*P*-value
Fluorescence	*P. australis*	**(Strains*P levels)+(Strains*N_Si levels)**					
		Strains	**0.08**	2	5534714	15.91	***2.30e-06***
		Plevels	**0.15**	2	10781859	30.99	***3.24e-10***
		N_Si levels	**0.22**	2	14221640	40.87	***2.83e-12***
		Strains: Plevels	**0.20**	4	16257595	23.36	***4.64e-12***
		Strains: N_Si levels	**0.20**	4	14260072	20.49	***5.18e-11***
		Residuals		66	11482387		
	P. fraudulenta	**(Strains*P levels)+(Strains*N_Si levels)**					
		Strains	**0.1**	2	22347145	24.716	***9.73e-09***
		P levels	**0.45**	2	99807909	110.387	***<2.0e-16***
		N_Si levels	**0.21**	2	46207225	51.105	***3.91e-14***
		Strains: P levels	**0.05**	4	11416094	6.313	***2.3 e-4***
		Strains: N_Si levels	**0.06**	4	12801508	7.079	***8.34 e-05***
		Residuals		66	29837286		
	P. pungens	**(Strains*P levels)+(Strains*N_Si levels)**					
		Strains	**0.02**	2	7424096	4.547	***0.0141***
		P levels	**0.35**	2	107508212	65.849	***<2e-16***
		N_Si levels	**0.40**	2	122012346	74.732	***<2e-16***
		Strains: P levels	0.02	4	6744340	2.065	0.0953
		Strains: N_Si levels	0.02	4	7238837	2.217	0.0766
		Residuals		66	53877665		
Total Domoic Acid	*P. australis*	**(Strains*P levels)+(Strains*N_Si levels)** *					
		Strains	**0.59**	2	0.8642	112.726	***<2e-16***
		P levels	**0.24**	2	0.4126	53.819	***1.37e-14***
		N_Si levels	0.01	2	0.0199	2.594	0.082330
		Strains: P levels	**0.06**	4	0.1006	6.562	***1.65e-4***
		Strains: N_Si levels	**0.04**	4	0.0703	4.585	***0.025***
		Residuals		66	0.253		
	P. fraudulenta	**(Strains*P levels)+(Strains*N_Si levels)** *					
		Strains	**0.03**	2	0.1078	3.172	***0.0484***
		P levels	**0.50**	2	1.8356	54.023	***<1.27e-14***
		N_Si levels	**0.10**	2	0.3784	11.136	***6.80e-05***
		Strains: P levels	0.04	4	0.1414	2.081	0.0932
		Strains: N_Si levels	0.03	4	0.115	1.692	0.1623
		Residuals		66	1.1213		
	P. pungens	**(Strains*P levels)+(Strains*N_Si levels)** *					
		Strains	0.02	2	1.02	1.567	0.2163
		P levels	**0.31**	2	17.913	27.537	***2.02e-09***
		N_Si levels	**0.19**	2	10.769	16.555	***1.49e06***
		Strains: P levels	**0.07**	4	3.855	2.963	***0.0259***
		Strains: N_Si levels	0.04	4	2.094	1.610	0.1822
		Residuals		66	21.466		
DDA	*P. australis*	**(Strains*P levels)+(Strains*N_Si levels)** *					
		Strains	**0.25**	2	3.69e-03	20.691	***1.06e-07******
		P levels	**0.21**	2	3.02e-03	16.966	***1.13e-06******
		N_Si levels	0.00	2	3.20e-05	0.177	0.83788
		Strains: P levels	0.05	4	7.35e-04	2.062	0.09573
		Strains: N_Si levels	**0.09**	4	1.33e-03	3.733	***8.47e-3****
		Residuals		66	5.88e-03		

* data transformed with "powertransform" application from Car package in R