INTRA- AND INTERSPECIES DIFFERENCES IN PSEUDO-NITZSCHIA GROWTH AND TOXICITY WHILE USING DIFFERENT NITROGEN SOURCES

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Submitted to Harmful Algae special issue on strain differences

ABSTRACT

Clonal cultures of plankton are widely used in laboratory experiments and have contributed greatly to knowledge of microbial systems. However, many physiological characteristics vary drastically between strains of the same species, calling into question our ability to make ecologically relevant inferences about populations based on studying one or a few strains. This study included nineteen non-axenic strains of three species of the diatom Pseudo-nitzschia isolated primarily from the mid-Atlantic coastal region of the United States. Toxin (domoic acid) production and growth rates were measured in cultures using different nitrogen sources (NH_4^+, NO_3^- and urea) and growth irradiances. The strains exhibited broad differences in growth rate and toxin content even between strains isolated from the same water sample. The influence of bacteria on toxin production was not investigated. Both P. multiseries clones produced toxin, yet preferentially used different nitrogen sources. Only two out of nine P. calliantha and two out of five P. fraudulenta isolates were toxic and domoic acid content varied by orders of magnitude. All three species had variable intraspecies growth rates on each nitrogen source, but P. fraudulenta strains had the broadest range. Light-limited growth rate and maximum growth rate in P. fraudulenta and P. multiseries varied with species. These findings show the importance of defining intra- and interspecies variability in ecophysiology and toxicity. Ecologically relevant functional diversity in the form of ecotypes or cryptic species appears to be present in the genus Pseudo-nitzschia.

Keywords: ammonia, diatom, domoic acid, nitrate, Pseudo-nitzschia, urea

INTRODUCTION

Much of what we know about plankton physiology comes from experiments using laboratory cultures. Most Pseudo-nitzschia culture studies focus on P. multiseries with the results extrapolated to other species and strains (Bates 1998). However, broad species and strain differences have been well documented (Gallagher 1980, 1982, Elrifi & Turpin 1985, Goldman & Dennett 1985, Holmes et al. 1991, Wood & Leatham 1992, Larsen & Bryant 1998, Burkholder et al. 2005) and draw into question the validity of making conclusions about microalgal species physiology based on one or a few strains.

Such high variability among strains casts doubt on the species concept in microalgae in general. While the creation of a species is the result of a biological reality, secondary characteristics are used (frustule morphology, ribosomal genes) to detect and define them. Manhart and McCourt (1992) stated that:

> "Practicing phycologists often seem to strive to delineate biological species while basing descriptions solely upon morphological data. The assumption is that morphological species closely approximate biological species of algae, but only rarely is this hypothesis tested. If species assignment is a hypothesis of relationship, then many (perhaps most) implicitly described biological species of algae represent untested hypotheses."

This implies a basic fault in microalgal species definitions which could explain high strain variability. However, Wood and Leatham (1992) argue that difficulties arise when culture studies involving only a few clones are used in an attempt to define interspecies differences without assessing within species variation, not necessarily as the result of a fault in the species

definition. The number of isolates necessary to accurately define within species variation using statistical methods is difficult to define and restricts the number of studies able to include such an analysis (Lakeman and Cattolico 2007).

The alternative, making conclusions about a species based on one strain, would likely lead to erroneous results. Toxin production in 17 strains of Alexandrium tamarense showed broad differences, the lowest approximating zero mouse units (MU) per 10^4 cells and the highest at 1.1 MU per 10^4 cells (Ogata et al. 1987). No one strain was representative of the species. Taking the analysis one step further, 15 sub-strains taken from one strain had a 0.6 MU per 10^4 cells range in toxin production. An analysis of PSP toxin composition in two strains of A. tamarense showed that one strain, SB31, produced mostly the sulfocarbamoyl derivative C2 while the other strain, SB32, produced mostly GTX3 and GTX4 (Cembella et al. 2002). Studies such as these show broad differences among strains and argue that finding one "representative" strain is highly unlikely if not impossible. Yet, an analysis of recent publications shows only 40% of studies that use culture experiments consider the possibility of significant strain differences when making conclusions (Burkholder & Glibert 2006).

Genetic variability in field populations and strains of Pseudo-nitzschia has been widely documented; however, physiological variability has not been as thoroughly investigated (Evans et al. 2004, Orsini et al. 2004). Many Pseudo-nitzschia culture studies present results from one strain (Bates et al. 1991, Douglas & Bates 1992, Hillebrand & Sommer 1996, Pan et al. 1996a,b, Fehling et al. 2004, Armstrong Howard et al. 2007). Others compare one strain of multiple species (Jackson et al. 1992, Hargraves et al. 1993, Wang et al. 1993, Maldonado et al. 2002,

Fehling et al. 2005). Most studies comparing multiple cultures of one species have focused on toxin production in P. australis, P. seriata or P. multiseries (Bates et al. 1989, Garrison et al. 1992, Douglas et al. 1993, Villac et al. 1993, Lundholm et al. 1994, Bates et al. 1999) with one study investigating toxicity in P. pseudodelicatissima (Pan et al. 2001). Only four studies used multiple strains of the same species to investigate other physiological processes in addition to toxicity. With four strains of P. multiseries and two strains of P. pungens, Bates et al. (1993) analyzed the effect of NO_3^- and NH_4^+ on growth and toxin production. Lundholm et al. (2004) employed two strains of P. multiseries to study the effect of pH on growth and toxin production. Bates et al. (1995) used three strains of P. multiseries to examine the role of bacteria in domoic acid (DA) production. Thessen et al. (2005) studied two strains of P. delicatissima, two strains of P. multiseries and three strains of P. pseudodelicatissima to assess the effect of salinity on growth rate.

Bacterial communities associated with algal cells are increasingly recognized as an important factor in cell physiology that can be highly variable between laboratory strains (Kaczmarska et al. 2005). In *Pseudo-nitzschia* cultures, biomass and growth rates are not affected by absence or reintroduction of bacteria, but DA production can be 2- to 95-fold higher in the presence of bacteria (Bates et al. 1995). Studies have verified that *Pseudo-nitzschia* is the source of DA and bacteria are incapable of producing DA autonomously (Douglas and Bates 1992, Bates et al. 2004). Bacteria play an as yet undefined role in DA production and can have a differential effect among strains and throughout the life of a culture (Stewart 2008). It has been proposed that the amount of DA measured in a culture is the result of competitive interactions between *Pseudo-nitzschia* production rate and utilization of DA by extracellular bacteria (Stewart 2008). This,

instead of genetic variability, could explain measured differences in DA production between *Pseudo-nitzschia* strains (Stewart 2008).

The meaning of high intraspecific genetic diversity in natural populations is controversial (Fenchel 2005, Foissner 2006). High genetic diversity in some protist taxa has been considered an indicator of cryptic species (species that are identical morphologically, but reproductively isolated) and functional diversity (Dolan 2005, Foissner 2006, Scheckenbach et al. 2006). Others argue that variation in rRNA is an accumulation of neutral mutations that does not correlate with physiology or show biogeographic patterns (Fenchel 2005). However, there have been populations of microalgae comprised of distinct physiological or genetic groupings which showed dynamic seasonal abundances. The presence of multiple ecotypes has been demonstrated in populations of Skeletonema costatum in Narragansett Bay (Gallagher 1980, 1982). These ecotypes have different physiological characteristics, making them better adapted to different environmental conditions and resulting in a succession of ecotypes throughout the year. Similar results have been found with Ditylum brightwellii in Puget Sound (Rynearson et al. 2006). Reproductively isolated cryptic species within P. delicatissima and P. pseudodelicatissima have been identified using morphology, genetic sequences and mating experiments (Amato et al. 2007). The ecological significance of this diversity is not well understood.

This paper is a presentation of strain differences between three species of Pseudo-nitzschia: P. multiseries, P. fraudulenta and P. calliantha for growth rate, toxin production, nitrogen use and

saturating growth irradiance. It is also the first presentation of genetic and toxin data from Chesapeake Bay area *Pseudo-nitzschia* strains.

MATERIALS AND METHODS

Culture isolation, identification and maintenance

Strains of Pseudo-nitzschia spp. were isolated from field samples via micropipetting (Andersen & Kawachi 2005) and incubated as separate cultures in an inorganic nutrient enriched seawater medium for diatoms, f/2* (Andersen et al. 1997), at a temperature and salinity close to ambient conditions at the time and place of collection and a 14:10 L:D cycle (Table 1). Morphological identification was performed using a derivation of methods in Lundholm et al. (2002a). A 10 mL aliquot of culture was preserved in 1% gluteraldehyde and digested using 2 mL of 30% H_2SO_4 and 10 mL of a saturated $KMnO_4$ solution in a 100 mL Erlenmeyer flask. The flask was covered with parafilm and swirled periodically throughout the digestion. After 24 h, a saturated oxalic acid solution was added until the sample became transparent. Samples were rinsed 3-4 times with distilled, deionized water and pelleted via centrifugation. The pellet was resuspended in approximately 1 mL of water. One drop was placed on a 200 μm mesh copper grid with a formvar film. Transmission electron microscopy (TEM) was used to identify species of Pseudo-nitzschia based on frustule morphology from measurements of cell properties in photographic negatives (Skov et al. 1999, Lundholm et al. 2002a,b, Lundholm & Moestrup 2002, Hasle & Lundholm 2005).

Genetic and phylogenetic analysis

DNA extractions from cultures were performed using the Puregene® DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Samples were centrifuged at 4000 g and the supernatants decanted. Pellets were resuspended in 300 µl of cell lysis buffer supplied with the kit and the manufacturer's protocol was followed for the remainder of the extraction procedure. Eluted DNA was stored at -20^{o} C until analyzed by polymerase chain reaction (PCR).

The nuclear encoded large subunit (D1-D3 region of the LSU; 28S) locus was amplified from *Pseudo-nitzschia* cultures using two primer sets. Primers D1R forward (Scholin et al. 1994; 5'-ACCCGCTGAATTTAAGCATA-3') and LSUSeqRev (5'-AGTGCTAGCAACAGACATCAACT-3') resulted in a 517 base pair amplicon, and D3Ca reverse (Scholin et al. 1994; 5'- ACGAACGATTTGCACGTCAG -3') and LSUSeqFor (5'-CTGAAACGGAAGCGAAGGAAA-3') resulted in a 434 base pair amplicon. The two amplicons overlapped by 144 base pairs.

The nuclear encoded internal transcribed region (ITS1-5.8S-ITS2) was amplified using two primer sets. Primers 1815F (Bowers et al. 2006; 5'-GGAAGTTGGGGGCAATAACAGG-3') and ITS Rev (Bowers et al. 2006; 5'-CATCGTTGTGGGAACCWAGACAT-3') were used to generate an amplicon from the first half of the ITS region, while ITS For (reverse complement of ITS Rev) and D1R Rev (reverse complement of D1R For mentioned above) were used to generate the second half. Amplicon sizes varied based on the species amplified. These two amplicons did not overlap, so species-specific primers were designed upstream of the ITSFor/Rev primer region to be used in conjunction with D1R Rev in order to generate overlapping sequences: *P. fraudulenta* For (5' – CGTTTGCCTCAAAAGTCAACTTG – 3'); *P.*

calliantha For (5' – TTTGGCTCGTGACTTTTGTTGC – 3'); and *P. multiseries* For (5' – TTGCCCGCCACTCTTTACGA – 3').

Each 50 µl PCR reaction contained 1.5 U of MegaFrag™ Taq polymerase (Denville Scientific, Metuchen, NJ); 10X PCR buffer and 4 mM $MgCl_2$ supplied with Taq polymerase; 2 mM each dNTP (Invitrogen, Alameda, CA), 0.25 mg mL^{-1} bovine serum albumin (Idaho Technology, Idaho Falls, ID), 0.8 µM each primer (Qiagen/Operon, Alameda, CA), 1 µl DNA template and molecular biology grade water to a final volume of 50 µl. Cycling was performed on the DNA Engine Dyad Peltier Thermocycler (Bio-Rad Laboratories, Inc., Waltham, MA) as follows: initial denaturation at 94°C for 2 min, followed by 45 cycles of 94°C for 10 s, annealing temperature ranging from 56°C to 60°C (based on primer pair used) for 30 s, extension step of 68°C for 30 s to 40 s (depending on amplicon size), and a final extension at 68°C for 6 min 20 s. PCR products were examined on a 1% ethidium bromide-stained agarose gel, and bands were extracted from the gel following the procedure supplied with the MinElute kit (Qiagen, Alameda, CA).

All primers outlined above were used for sequencing amplicons. Gel-extracted bands were sequenced using the DYEnamic™ ET Terminator Cycle Sequencing kit (Amersham Biosciences, Piscataway, NJ). The sequencing reactions contained the following: 2 µl dye (diluted 1:5), 1 µl of desired primer (0.4 µM final concentration), 0.5 - 1 µl of gel purified product, and sterile H_2O to 5 µl. Cycling parameters were as follows: 25 cycles of 95°C for 20 s, 55°C for 15 s and 60°C for 1 min. After cycling, sequencing reactions were centrifuged through Sephadex G50 to remove unincorporated dye (Amersham Biosciences, Uppsala, Sweden). Sequencing was

performed on the 3100 capillary sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned and inspected for nucleotide ambiguities using Sequencher (version 4.1.2, Gene Codes Corporation, Ann Arbor, MI) and then aligned to other Pseudo-nitzschia species available from GenBank utilizing the software MacClade (version 4.04; Maddison & Maddison; Sinauer Associates, Inc., Sunderland, MA). The BLAST search program available through the National Center for Biotechnology Information website (www.ncbi.nih.gov/) was used to determine the closest sequence matches available in GenBank (Altschul et al. 1997). Parsimony analysis was performed using PAUP* 4.0b10 (Swofford 2002).

Toxin Content of Cultures

Potential toxicity of each culture was tested in f/2* medium with 53 μM Si (to induce Si limitation during stationary phase of growth; Bates et al. 1991, Pan et al. 1991) with bubbling at an irradiance approximating 150 μmol photons m^{-2} sec^{-1} and a 14:10 L:D cycle in 15°C (Bates 1998). In vivo fluorescence of a subsample was measured at the same time daily to monitor growth phase and timing of toxin sample collection. An emphasis was placed on documenting DA concentrations in exponential and stationary phases at least one week after peak fluorescence to capture maximum intracellular toxin (Bates 1998). For the toxin sample, 50 mL of culture was removed and filtered through a Whatman GF/F filter. Both the filter and filtrate were stored at -80°C in the dark for determination of particulate and dissolved toxin. DA is synthesized in the cell and released in variable amounts that increase as the culture ages (Bates et al. 1991, Maldonado et al. 2002). Therefore, both fractions are necessary for quantifying DA production, especially in late stationary phase, when the dissolved fraction is highest (Bates et al. 1991). Care was taken to filter at a low vacuum pressure (< 130 mm Hg) to avoid breaking cells and

thus overestimating the dissolved fraction (Bates et al. 1998). Samples were analyzed by high performance liquid chromatography (HPLC; Quilliam 2003) or the ELISA method (Garthwaite et al. 1998) using the ASP direct cELISA test kit (Biosense, Bergen, Norway). HPLC analysis was used to quantify particulate DA initially, but the limit of detection (375 pg DA mL^{-1}) was too high for many samples. Toxin content in one strain of P. multiseries (Pn-1) and four strains of P. calliantha (Pn-4, Pn-6, Pn-7 and Pn-8) was determined with this method. The ELISA method has lower limits of detection (9.5-388 pg mL^{-1}) and provided results for more samples, but can have higher variation between replicates. Individual kits have their own quantitation limit (LOQ) and detection limit (LOD = 1/3 of LOQ). Concentrations that are below the LOQ, but above the LOD are considered semi-quantitative. The test is positive for DA, but the accuracy is less than if the concentration had been above the LOQ. Since each test kit can process a maximum of 36 samples, not all samples from the same culture could always be processed on the same plate and therefore have the same LOD and LOQ (Table 2).

A preserved sample (1 % gluteraldehyde final concentration) for cell counts was taken with the toxin sample. An aliquot of each preserved sample was stained with 0.03% proflavin hemisulfate, filtered onto a 2 μm polycarbonate filter, and mounted on a glass slide with immersion oil for enumeration via epifluorescent microscopy (excitation 450-490 nm, dichromatic beam splitter 500 nm, barrier filter 515 nm; Nikon filter set EF-4 B-2A) at 400X magnification.

Varying Nitrogen Source

To test the effect of nitrogen on growth rate and toxin production, each strain was adapted to grow in f/2* with one nitrogen source, NO_3^-, NH_4^+, or urea, and Si at f/20 (88 µmol N, 11 µmol Si) concentrations at 15°C with 150 – 200 µmol photons m^{-2} sec^{-1} (14:10 L:D cycle) through two batch cultures as in previous studies (Thessen et al. 2005). When the cultures were adapted, five replicate 125 mL flasks were inoculated. Chlorophyll a was measured at the same time daily by in vivo fluorescence of a subsample of the flasks and used to calculate specific growth rates (Wood et al. 2005). Toxin and preserved samples were taken one week after the beginning of stationary phase to compare toxin production and cell abundance. Dissolved DA $cell^{-1}$ was calculated by dividing dissolved DA by cell abundance.

Differences in growth rates on each nitrogen source were determined for each strain by one-way ANOVA using Tukey's studentized range test (SAS®, version 9.1, SAS Institute, Inc., Cary, NC, USA) at the 5% level. Overall differences among all treatments were analyzed with a model I one-way nested ANOVA with a type III sums of squares where replication was uneven (SPLUS®, version 6, Insightful Corporation, Seattle, WA, USA). Variation was determined within nitrogen source, species, strain and error by parsing the sum of squares. Identical statistical analysis was performed on the toxin data. Correlation between growth rate and toxin content was analyzed using Spearman's correlation coefficient (SPLUS®, version 6). Replication is given in Table 3.

Growth versus Irradiance

Three strains of two species were adapted through two batch culture generations to the experimental temperature (10°C, 15°C or 20°C) and irradiance (20 µmol photons m^{-2} sec^{-1} to 500

μmol photons m^{-2} sec^{-1}). The experiment was performed in f/2* media at a salinity of 32 in five replicate 10 mL glass tubes at each discrete irradiance. Treatments started at 20 μmol photons m^{-2} sec^{-1} with a 14:10 L:D cycle and increased at approximately 50 μmol photons m^{-2} sec^{-1} intervals until growth rate saturation. Cultures were monitored daily using in vivo fluorescence. Growth rates were calculated as described above. Replicates were averaged and a standard deviation was calculated. The data were fitted to a non-linear least squares regression using an equation initially derived for photosynthesis (Platt et al. 1980) and modified to describe growth:

$$\mu = \mu_o + \mu_{max}(1 - e^{((-E\alpha)/\mu max)})e^{((-E\beta)/\mu max)}$$

where μ_o is the y intercept, μ_{max} is the maximum potential growth rate, α is the initial light-limited slope, E is the irradiance and β is the slope of the photoinhibited part of the curve. E_k, the irradiance saturating for growth, was calculated for each temperature treatment by dividing μ_{max} by α. Significant difference between treatments for each regression parameter was calculated using a Gabriel Approximation (Sokal & Rohlf 1995).

RESULTS

Identification of Cultures

Eighteen of nineteen cultures were identified as one of three species: P. multiseries, P. calliantha or P. fraudulenta (Fig. 1; Table 1). Morphometric measurements of the frustules fell within previously reported values for each species (Table 4). ITS and LSU rRNA sequences of the cultures showed identical or close relationships to sequences deposited on GenBank from strains of the same morphological species isolated globally. The P. multiseries culture Pn-1 had 100% LSU sequence similarity to three strains (NWFSC005 and NWFSC011 from Washington and OFPm984 from Japan) and was two base pairs different from a California isolate CV19 (Fig.

2A). Pn-1 had 100% ITS sequence similarity to California strain mu3 and was two base pairs different from the Japanese strain OFPm984 (Fig. 2B). Table 1 provides GenBank accession numbers for LSU and ITS sequences derived from Chesapeake Bay isolate Pn-1.

Sequences for P. fraudulenta cultures Pn-9, Pn-10, Pn-11, Pn-12 and Pn-15 were identical for both the ITS and LSU loci. LSU sequences were identical to those of a strain from Spain, Limens1, and less than ten base pairs different from three strains (SZN-B21, SZN-B40 AND SZN-B22) from Italy (Fig. 2A). The ITS sequence data was two base pairs different from the Spain Limens1 isolate (Fig. 2B). Table 1 provides GenBank accession numbers for LSU and ITS sequences derived from Chesapeake Bay isolates Pn-9, Pn-10, Pn-11, Pn-12 and Pn-15.

LSU sequences for P. calliantha cultures Pn-2, Pn-3, Pn-4, Pn-6, Pn-7, Pn-8 and Pn-13 were identical, while the ITS sequences exhibited polymorphisms at two positions. Although there were no P. calliantha sequence data available for the LSU locus on GenBank, BLAST results showed that the LSU sequence was closest to thirteen P. pseudodelicatissima isolates (P-11 from Portugal, NWFSC047, NWFSC040 and NWFSC006 from Washington and the remaining isolates from Italy). There was high genetic variability between all of these isolates, and our sequence showed approximately 0.7 - 5% divergence from those sequences (Fig. 2A). Our ITS sequences shared 99% sequence similarity to three P. calliantha isolates from Vietnam (Fig. 2B). Table 1 provides GenBank accession numbers for LSU and ITS sequences derived from Chesapeake Bay isolates Pn-2, Pn-3, Pn-4, Pn-6, Pn-7, Pn-8 and Pn-13.

Toxicity of Cultures

All batch cultures increased their abundance by at least ten-fold during this experiment. Lag phase, growth rate and time to onset of stationary phase varied. Out of 16 strains tested, seven produced domoic acid (Table 5). All three species cultured had at least two toxic isolates. DA content data is shown normalized to volume (DA mL^{-1}) and cell number (DA $cell^{-1}$) for all experiments.

The P. multiseries isolates had the two highest DA concentrations, per mL and per cell (Figs. 3, 4). P. calliantha and P. fraudulenta isolates produced between 10^3 and 10^2 pg DA mL^{-1} (between 1 and 10^{-4} pg DA $cell^{-1}$) with P. calliantha producing the least (Pn-3) and the most (Pn-8) toxin per mL (Fig. 3). Per cell, P. fraudulenta (Pn-12) produced the most (0.16 pg DA $cell^{-1}$) and P. calliantha (Pn-3) produced the least ($2.62X10^{-4}$ pg DA $cell^{-1}$) amount of toxin (Fig. 4). Growth rates and maximum abundances of strains that did not produce DA were not different from isolates that did produce DA (Table 5). Two of the three species cultured, P. calliantha and P. fraudulenta, had non-toxic isolates (Table 5). Three strains of P. calliantha (Pn-2, Pn-5 and Pn-18) died within months of isolation, before toxin content was investigated.

The highest total DA levels occurred during stationary phase in all strains. This pattern holds in axenic and non-axenic cultures (Bates et al. 2004). However, in P. multiseries and one P. fraudulenta strain (Pn-12), some DA was also present before stationary phase while the culture was still growing (Figs. 3, 4). The two P. multiseries strains (Pn-1, CLN47) show similar DA production patterns, with DA present throughout the entire growth cycle, gradually increasing as cell division slowed and reaching a maximum in stationary phase. The two P. calliantha strains (Pn-3, Pn-8) also showed similar DA production patterns with DA produced only once the

cultures had stopped growing. The two P. fraudulenta strains (Pn-12, Pn-9) showed different DA production patterns. One strain (Pn-12) produced DA during late exponential phase while the other (Pn-9) did not produce toxin until stationary phase. DA is a relatively stable molecule under laboratory culture conditions (Bouillon et al. 2006), thus high DA content of the first sample in some strains (Pn-1, CLN47 and Pn-12) is probably carry-over from the inoculum used to start the culture and does not signify any real production of DA in those cultures at the beginning of growth.

Varying Nitrogen Source and Growth Rate

Nitrogen source (NH_4^+, NO_3^- and urea) affected growth rates in Pseudo-nitzschia cultures, but not in a way that could be predicted based on nitrogen source and species (Fig. 5; Table 6). Statistical analysis revealed significant growth rate differences between nitrogen source, species and strain with strain being the highest source and replication the lowest source of variability (Table 6). The two P. multiseries strains had different nitrogen responses, with Pn-1 growing fastest on NO_3^- and NH_4^+ (0.75 d^{-1}) while CLN47 grew equally well (0.6 d^{-1}) on all sources. The five P. fraudulenta strains showed three different growth responses with Pn-10 and Pn-12 growing fastest on NO_3^- and NH_4^+ (0.74-0.88 d^{-1}), while Pn-11 and Pn-15 grew fastest on NH_4^+ (1.21 d^{-1} and 1.16 d^{-1} respectively) and Pn-9 grew fastest on urea (1.02 d^{-1}). The two P. calliantha strains had different growth responses, but similar growth rates. Pn-13 grew fastest on NO_3^- and NH_4^+ (0.7 d^{-1}). Pn-8 grew fastest on NO_3^- (0.86 d^{-1}). Nitrogen was an important source of variation in growth rate, but growth was also significantly affected by strain.

Strains of P. multiseries (Pn-1), P. fraudulenta (Pn-10) and P. calliantha (Pn-13), despite being different species, showed similar relative growth responses and growth rates. P. fraudulenta strains isolated from the same water sample (Pn-9, Pn-10, Pn-11 and Pn-12) had the largest differences in relative growth response and growth rate. The only trend in all of these data is higher growth rates on NH_4^+ and lower growth rates on urea, with the exception of one P. fraudulenta culture (Pn-9).

Varying Nitrogen Source and Toxicity

DA concentrations were affected by nitrogen source just as unpredictably as growth rate (Figs. 6, 7; Table 6). Statistical analysis revealed significant differences in toxin content between nitrogen source, species and strain with strain being the highest source of variability in DA content $cell^{-1}$ and mL^{-1}, while for particulate DA mL^{-1} the highest source of variability is species (Table 6). The only insignificant effect was that of nitrogen on total DA $cell^{-1}$. Two strains of P. multiseries produced the most DA while growing on NH_4^+ (Pn-1, 3984 fg $cell^{-1}$) and NO_3^- (CLN47, 369 fg $cell^{-1}$; Tables 5, 7; Figs. 6, 7) and contained the highest total toxin of all strains tested. DA concentration in one strain of P. calliantha (Pn-8) and one strain of P. fraudulenta (Pn-9) did not vary significantly with nitrogen source. One strain of P. fraudulenta (Pn-12) contained more DA while growing on NH_4^+ (23.7 fg $cell^{-1}$) and urea (43.3 fg $cell^{-1}$) than NO_3^- (3.1 fg $cell^{-1}$). The other P. fraudulenta strain (Pn-9) contained more particulate toxin when growing on NO_3^- (0.29 fg $cell^{-1}$). Only the two P. multiseries (Pn-1 and CLN47) strains always produced detectable DA. This is probably due to the P. calliantha and P. fraudulenta strains having low toxin content, near the detection limit of the assay, leading to a high error term in the results.

Growth rates and toxin production did not track each other except in P. multiseries (Pn-1), where the highest growth rate (0.75 d^{-1}) accompanied the highest toxin content (3984 fg $cell^{-1}$). Total DA, particulate DA and dissolved DA varied with nitrogen source in both of the P. multiseries strains (CLN47, Pn-1) and one of the P. fraudulenta strains (Pn-12), with no real pattern or relationship to growth rate (Figs. 8, 9). For example, the highest DA content in CLN47 (371 fg $cell^{-1}$) occurred in the NO_3^- treatment, but growth rate was neither the highest nor the lowest on this nitrogen source. This suggests that nitrogen source has an effect on DA production besides the direct effect on growth rate. Differences in the pattern of toxin content between data given as ng DA mL^{-1} (Fig. 8) and fg DA $cell^{-1}$ (Fig. 9) were due to differences in cell number mL^{-1} at time of sampling and the change from nanograms to femtograms in the unit.

Statistical analysis revealed no significant correlation between growth rate and toxin content for CLN47, Pn-8 and Pn-12 (Table 8). However, growth rate was correlated with dissolved DA normalized to cell number and volume and total DA normalized to cell number in strain Pn-9. Growth rate in Pn-1 was strongly correlated with particulate, dissolved and total DA normalized to both cell number and volume. Toxin content normalized to cell number and volume were nearly identical. CLN47, Pn-1 and Pn-8 showed the same pattern in DA content per cell and per mL. One P. fraudulenta strain (Pn-12) contained more particulate DA per mL while growing in NH_4^+ and urea with no significant difference in dissolved DA (0.06-1.18 ng mL^{-1}). Per cell, there was no significant difference in particulate DA (0-0.78 fg $cell^{-1}$).

Growth versus Irradiance

Growth versus irradiance experiments were performed on three strains of two species at three temperatures (Fig. 10). P. fraudulenta (Pn-15) would not grow at all light levels at all temperatures; therefore the curve at 20°C is incomplete. Curve parameters α (d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$)† and μ_{max} (d^{-1}) showed significant differences between species at the same temperature (Table 9). At 10°C, α (0.0263 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$) in P. fraudulenta was different from α in the *P. multiseries* strains (Pn-1 and CLN47) which were not different from each other (0.0067, 0.0127 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$ respectively). At 15°C, α in P. fraudulenta (0.0239 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$) was different from only one of the P. multiseries strains (0.0047 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$, Pn-1) which were not different from each other (0.0109 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$, CLN47). At 20°, α was not significantly different between strains of P. multiseries (0.0094 - 0.0098 d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$; Table 9). Alpha was not significantly different between temperatures within the same strain. At 10°C, μ_{max} (d^{-1}) did not show any difference between strains. At 15°C, however there was a difference in μ_{max} between P. fraudulenta (0.68) and one of the P. multiseries strains (0.33, CLN47). There was no statistically significant difference between μ_{max} at 20°C (0.78-2.0), probably due to the high variability in P. multiseries (Pn-1). None of the strains showed significant changes with temperature. Comparisons of β were not possible because not all strains were photoinhibited. Saturating irradiance (E_k) was calculated for each treatment where possible, but had such high variability that none of the data were significant (Table 9).

DISCUSSION

† Unit for α reduces to µmol photons m^{-2}*86400. While conceptually correct, this method uses unusual units because α is typically used to describe P vs. E curves instead of µ vs. E curves.

Sequences for the LSU (large subunit of the ribosome) and ITS (internal transcribed spacer) regions were successfully combined with morphology to identify Pseudo-nitzschia strains to species level. Both morphological and genetic data were conclusive and consistent for identification of P. multiseries, P. fraudulenta and P. calliantha, indicating an absence of cryptic or sibling species within those groups. However, these isolates were probably only a small representation of a more genetically diverse Pseudo-nitzschia population in the Chesapeake Bay. In fact, real-time molecular assays designed against the three species isolated from the Bay only identified these species in a small percentage of environmental water samples where Pseudo-nitzschia had been identified using light microscopy (data not shown). Field studies using rRNA sequences and microsatellite markers have found high genetic diversity in Pseudo-nitzschia populations (Orsini et al. 2004, Evans et al. 2005). The high divergence between strains of P. calliantha and the P. pseudodelicatissima in GenBank (0.7-5%) is indicative of the pseudodelicatissima species complex that was recently partially resolved based on morphology and genetics (Lundholm et al. 2003) and may still need further resolution. The lack of such variability in Pseudo-nitzschia cultures world-wide could be an artifact of the culturing process favoring strains with similar genetics (Ward et al. 1990, Amann et al. 1995). Alternate locus sequencing or microsatellite analysis could reveal more diversity in cultures. Physiological parameters tested in this study were not consistently related to species, suggesting the presence of ecotypes and high functional diversity in the species tested. Genetic and functional diversity among and within Pseudo-nitzschia species needs to be explored further in order to gain a better understanding of the strains present and how they are related to natural populations.

In batch culture, Pseudo-nitzschia typically produces DA when in stationary phase, once cell division has stopped and the culture is limited by P or Si, for example, with replete N and light (Bates 1998). This is seen in Pn-1, Pn-3, Pn-8 and Pn-9 as well as in other studies with P. multiseries (Subba Rao et al. 1990, Bates et al. 1991, 1993, 1995, Douglas & Bates 1992; Wohlgeschaffen et al. 1992, Douglas et al. 1993, Whyte et al. 1995, Kotaki et al. 1999, Lundholm et al. 2004), P. seriata (Lundholm et al. 1994, Fehling et al. 2004) and P. australis (Cusack et al. 2002). In CLN47 and Pn-12, there is evidence of toxin production during late exponential phase, similar to results for P. pseudodelicatissima (Pan et al. 2001), P. australis (Garrison et al. 1992) and for P. multiseries (Pan et al. 1996a). A possible explanation for DA production in late exponential phase, before the culture has stopped growing, is that this phase may be a period of transition, when some cells have stopped dividing and are producing DA while others are still growing (Pan et al. 1996a, Bates 1998). High DA content during lag phase is probably from the inoculum used to start the culture; however, it is possible that the shock of being transferred led to DA production in lag phase cultures (Bates 1998). It is also generally assumed that particulate DA peaks about one week after the beginning of stationary phase, then decreases as dissolved DA increases (Bates 1998). Total DA content did not peak in any of our cultures within the 26 d duration of the experiment, with the exception of Pn-12.

It is generally thought that NH_4^+ is more readily taken up by phytoplankton than NO_3^- due to its inhibition of nitrate reductase and lower energy requirement for use (McCarthy et al. 1977, Losada & Guerrero 1979, Syrett 1981). However, studies have shown that inhibition of NO_3^- uptake is rarely as complete or as common as sometimes believed (reviewed in Dortch 1990). Uptake and reduction of NO_3^- can be decoupled due to the much lower amount of energy

required to take up NO_3^- compared to reducing NO_3^-. When temperatures are low, nitrate reduction enzymes are limited (Lomas and Glibert 2000). Diatoms in particular take up large amounts of NO_3^- under high light, low temperature conditions, resulting in large internal pools (DeManche et al. 1979, Dortch et al. 1979, Collos 1982, Lomas & Glibert 1999, 2000). Diatoms are known to grow equally well on NH_4^+ and NO_3^- (Eppley & Renger 1974) and studies on phytoplankton assemblages show utilization of whatever nitrogen source is readily available (McCarthy et al. 1977, Dortch 1990). Uptake rates of NO_3^-, NH_4^+ and urea by diatoms vary considerably and depend on the physiological state of the cell, but uptake of all three nitrogen species in diatoms has been documented (Eppley et al. 1969, McCarthy 1972).

These results demonstrate the ability of Pseudo-nitzschia to grow and become toxic on multiple nitrogen sources. P. multiseries can grow on NH_4^+, NO_3^-, urea, glutamine and NO_2^- with NH_4^+ supporting the slowest growth (Hillebrand & Sommer 1996). NH_4^+ concentrations above 200 μM do not support growth due to NH_4^+ toxicity (Hillebrand & Sommer 1996), and only at NH_4^+ concentrations of 55 - 110 μM were growth rates comparable to those in NO_3^- (Bates et al. 1993). The present study used 88 μM nitrogen. Growth rates (d^{-1}) for P. multiseries were slightly less (0.36 - 0.33 for 300 μM NH_4^+, 0.42 – 0.55 for 200 μM NO_3^- and 0.38 – 0.60 for 200 μM urea) than those in our experiments (0.61 – 0.76 for NH_4^+, 0.45 – 0.8 for NO_3^- and 0.3 – 0.68 for urea; Hillebrand & Sommer 1996). A study of cultured P. australis (using 50 μM nitrogen) showed equal growth on NO_3^- and NH_4^+, but reduced growth on urea, with the latter culture containing the highest DA (Armstrong Howard et al. 2007). This is unlike any of the present cultures, except Pn-12 (P. fraudulenta). Another study showed higher DA content in P. multiseries treated with 440 μM NH_4^+ than 440 μM NO_3^- (4.2 pg $cell^{-1}$ in NH_4^+ and ~1.6 pg cell$^-$

[1] in NO_3^- ; Bates et al. 1993) which is comparable to the DA content in P. multiseries (Pn-1) in our study (4.0 pg $cell^{-1}$ when grown in NH_4^+ and 1.9 pg $cell^{-1}$ when grown in NO_3^-). These data show that specific growth rate and toxin content of Pseudo-nitzschia can be affected by nitrogen source, but cannot be predicted based on nitrogen and species. There is clearly no strain in this study that is representative of the growth response or toxin content of the genus or any of the three species.

There are two important concerns with the toxin samples for the nitrogen experiments. First, even though all cultures were sampled at the same time relative to their growth phase, one week after the fluorescence peak is not enough time for these strains to reach maximum DA content (Fig. 3). Some of the variation between treatment, species and/or strain could be due instead to time of sample collection relative to DA production cycle in batch culture. Second, working with very low amounts of DA on multiple ELISA plates could have resulted in the unusual zero values in P. fraudulenta (Pn-12) and P. calliantha (Pn-8).

Statistical analysis (ANOVA) revealed that most variation in toxin content and growth rate is due to strain (except particulate DA mL^{-1}), arguing that strain is more important than species in defining the physiology of Pseudo-nitzschia. However, species was the second most important source of variation. If more species were included in this study, especially more toxic species, this relative importance could shift so that species is more important than strain. The actual treatment, nitrogen source, resulted in the lowest source of variation in toxin content and growth rate, sometimes lower than the replicate cultures (particulate DA and total DA $cell^{-1}$), but it was responsible for a higher proportion of variation in growth rate than in toxin content. Nitrogen

source had more of an effect on particulate and total DA mL^{-1} than DA $cell^{-1}$, suggesting that the affect of nitrogen source on toxin content is an indirect effect of growth rate on cell abundance.

Most of the variation in toxin content in this study can be attributed to strain differences, indicating that intraspecies variation is larger than interspecies variation especially among low toxin-producing species. In this context "strain differences" can be genetic differences or differences in bacterial flora within the culture. We did not address variation in epibiotic bacteria, a potential source of variation in toxin levels. For example, two cultures of the same strain (CLN-1 and CLN-1 NRC) of P. multiseries that had been maintained in separate laboratories for 2 years differed in their epibiotic bacteria and toxin production (Kaczmarska et al. 2005). The culture with more diverse bacterial flora produced the most DA (CLN-1). It is unlikely that changes in DA production within batch cultures over the growth cycle are due to bacteria because both axenic and non-axenic cultures show the same pattern of increased DA production in stationary phase (Bates et al. 1995). It is also unlikely that differences in DA content between nitrogen treatments in this study are due solely to bacteria because of the shorter time passed between subculturing and experimentation (months rather than years). However, differences in toxin content between strains of the same species in this study could be affected by bacteria.

Previous studies showed a relationship between growth rate and DA content using one strain of P. multiseries in continuous and batch culture (Pan et al. 1996a,b). As growth rate increased in continuous cultures, DA content decreased. This study found an inverse relationship between growth rate and toxin content only in Pn-9 (Table 8). Growth rate and toxin content in Pn-1 was

found to be positively correlated and no relationship was found in the other strains. However, these experiments were performed on stationary phase batch cultures, which are different from slowly growing continuous cultures.

The growth versus irradiance curves seem to show differences in α and μ_{max} among temperature treatments (Fig. 10). However, high variability associated with the 20°C treatments render these differences statistically insignificant (Table 9). Using a turbidostat to calculate a growth vs. irradiance curve (Falkowski et al. 1985) instead of using replicate batch cultures at discrete irradiances could result in less error. Replication could be achieved by collecting data for multiple curves instead of replicates of points on a single curve. Only one other study contains μ vs. E data for Pseudo-nitzschia (Pan et al. 1996b), where an α of 0.0027 d^{-1} [μmol photons m^{-2} sec^{-1}]$^{-1}$ was observed at 10°C in the P. multiseries culture NPBIO, but the curve is not very well described below 200 μmol photons m^{-2} sec^{-1}, the light-limiting part of the curve in that study (Fig. 10). Nevertheless, the α is within the range observed for P. multiseries in the present experiments (Table 9). The incomplete curve at 20°C for Pn-15 (Fig. 10) can be explained by intolerance of this strain to higher temperatures; this strain would not adapt to 20°C at low irradiance. Pn-15 was isolated from cold waters (Table 1) and could represent an ecotype that is adapted to survive at low temperatures. Pn-15 had its highest growth rates at 10°C (Fig. 10). A cold-water strain of P. granii also did not grow below 100 μmol photons $m^{-2}sec^{-1}$ at 20°C (El-Sabaawi & Harrison 2006). Chlorophyll in these P. granii cells decreased above 14°C suggesting that the decline in growth rate at lower irradiance may have been due to an inability to capture adequate light to support increased enzyme activity associated with higher temperatures (El-Sabaawi & Harrison 2006).

There was concern that results might be affected by differences over time in culture as many physiological properties have been known to change with culture age, especially Pseudo-nitzschia toxin production (Bates et al. 1999). As an individual batch culture of harmful algae ages and approaches stationary growth toxin production typically increases (Bates et al. 1998, Dias et al. 2002). Additional parameters such as growth rate, chlorophyll fluorescence and lipid composition are known to increase or decrease throughout the growth cycle of a batch culture, but often return to previous values when a new batch culture is started (Mansour et al. 2003, Liang et al. 2006). Long-term physiological changes are likely to occur when a strain is held in the laboratory for several years. Pseudo-nitzschia strains typically lose toxicity the longer they are grown in the laboratory, possibly due to interactions with bacteria flora (Stewart 2008), which can change as the strain ages (Kaczmarska et al. 2005). Most of the cultures were approximately the same age (one year) during this study (Table 10).

Results do support the following: Defining diatom species based on morphology alone can be inadequate. A more thorough approach is required which includes morphological investigations, genetic sequencing, mating experiments and physiological experiments (Mann 1999). Numerous Pseudo-nitzschia spp. have been described by combinations of morphology and gene sequences (Lundholm et al. 2002a,b, 2003, 2006, Lundholm & Moestrup 2002). Only one Pseudo-nitzschia study combines morphology, genetic sequencing and mating experiments (Amato et al. 2007). As the impact of strain differences is recognized, diatom studies should include an investigation of the biological and phylogenetic species concepts in addition to the more traditional morphological species concept (e.g., Behnke et al. 2004, Mann et al. 2004).

The ability of Pseudo-nitzschia to grow under multiple nitrogen, light and temperature conditions indicates a broad ecological niche, which reflects the cosmopolitan nature of the genus. Not all strains were able to grow optimally under all treatments, but at least one strain did grow optimally under all conditions tested. This, in combination with high genetic variability in natural populations (Evans et al. 2005), suggests that Pseudo-nitzschia is able to take advantage of multiple regimes in a changing environment. For example, a species of *Pseudo-nitzschia* might be present in a system throughout the spring and appear to be static, but genetic or physiological analyses would reveal a dynamic succession of subpopulations as environmental parameters changed and favored different ecotypes. These data suggest the existence of multiple ecotypes in natural populations of Pseudo-nitzschia, similar to Skeletonema costatum and Ditylum brightwellii (Gallagher 1982, Rynearson et al. 2006). Since all of the strains tested in this study came from the same region, nothing can be concluded about biogeography; however, it would be reasonable to investigate strains from multiple regions as Pseudo-nitzschia is a cosmopolitan diatom genus (Hasle 2002).

ACKNOWLEDGEMENTS

The authors would like to thank S. S. Bates for use of his P. multiseries culture, CLN47, and SEM micrographs, E. Gantt for assistance with electron microscopy, D. Caron and A. Schnetzer for assistance with the ELISA procedure, P. M. Glibert, K. G. Sellner and J. M. O'Neil for commenting on early drafts and two anonymous reviewers for their valuable editorial comments. This research was supported by EPA STAR fellowship program FP916343 (A.E.T) and funding

through the Centers for Disease Control (H.A.B); DHMH OCPMP 048315G/M00B400298. This manuscript has not been formally reviewed by the EPA. The views expressed in this document are solely those of the author, and the EPA does not endorse any products or commercial services mentioned in this publication. This is UMCES contribution No. 4244.

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Table 1**:** Cultures of Pseudo-nitzschia spp. used in the present study.

Clone	Species	GenBank Accession #		Collection Site Information[a]			
		ITS	LSU	Station	Date	Temp (°C)	Salinity
Pn-1	*Pseudo-nitzschia multiseries*	DQ445651	DQ445638	Choptank River	11/18/2002	11	17
Pn-2	*Pseudo-nitzschia calliantha*	DQ445652	DQ445639	Choptank River	4/28/2003	15	9
Pn-3	*Pseudo-nitzschia calliantha*	DQ445653	DQ445640	Choptank River	4/14/2003	10	11
Pn-4	*Pseudo-nitzschia calliantha*	DQ445654	DQ445641	Chesapeake Bay	10/3/2003	21	17
Pn-5	?			Chesapeake Bay	10/3/2003	21	17
Pn-6	*Pseudo-nitzschia calliantha*	DQ445655	DQ445642	Chesapeake Bay	10/4/2003	20	12
Pn-7	*Pseudo-nitzschia calliantha*	DQ445656	DQ445643	Patuxent River	10/13/2003	20	11
Pn-8	*Pseudo-nitzschia calliantha*	DQ445657	DQ445644	Choptank River	10/20/2003	17	10
Pn-9	*Pseudo-nitzschia fraudulenta*	DQ445659	DQ445645	Assateague Island	3/28/2004	6	32
Pn-10	*Pseudo-nitzschia fraudulenta*	DQ445660	DQ445646	Assateague Island	3/28/2004	6	32
Pn-11	*Pseudo-nitzschia fraudulenta*	DQ445661	DQ445647	Assateague Island	3/28/2004	6	32
Pn-12	*Pseudo-nitzschia fraudulenta*	DQ445662	DQ445648	Assateague Island	3/28/2004	6	32
Pn-13	*Pseudo-nitzschia calliantha*	DQ445658	DQ445649	Choptank River	5/17/2004	23	9
Pn-14	*Pseudo-nitzschia calliantha*			Kiptopeke Virginia	11/11/2004	14	30
Pn-15	*Pseudo-nitzschia fraudulenta*	DQ445663	DQ445650	Asilomar California	10/7/2005	11	32
CLN47	*Pseudo-nitzschia multiseries*			CL147 X CL191[b]	6/16/2005		
Pn-16	*Pseudo-nitzschia calliantha*	EF621757	EF621754	Tangier Sound	11/14/2006	13	15
Pn-17	*Pseudo-nitzschia calliantha*	EF621758	EF621755	Tangier Sound	11/14/2006	13	15
Pn-18	*Pseudo-nitzschia calliantha*	EF621759	EF621756	Tangier Sound	11/14/2006	13	15

[a]Collection site information column contains data on water samples from which cultures were isolated.
[b]Strain CLN47 was isolated after sexual reproduction between strains CL147 and CL191.

Table 2: Limit of quantitation and limit of detection for ELISA method used to analyze data presented in Figs. 3 and 4. Particulate DA for Panel A and Panel C were obtained via HPLC, thus no LOD is presented.

Panel	Number	particulate		dissolved	
		LOQ	LOD	LOQ	LOD
A	1	375		345	115
B	1	163.2	54.4	1020	340
	2	28.8	9.6	1020	340
	3	792	264	1020	340
	4	792	264	396	132
C	1	375		420	140
	2	375		1020	340
	3	375		1165	388.3
D	1	111.2	37	1165	388.3
	2	28.4	9.47	420	140
E	1	55.2	18.4	345	115
	2	44.8	14.9	345	115
	3	55.2	18.4	1020	340
	4	28.8	9.6	1020	340
F	1	111.2	37	420	140
	2	34	11.3	420	140

Table 3**:** Replicates (n) for experiments testing growth and toxin production in cultures grown on different nitrogen sources. Data are in Figures 5, 6 and 7.

Strain	Species	toxin			growth		
		NO_3^-	NH_4^+	urea	NO_3^-	NH_4^+	urea
Pn-1	***P. multiseries***	**5[a]/5[b]**	**5/5**	**5/5**	**5**	**5**	**5**
Pn-3	***P. calliantha***				**4**	**5**	**5**
Pn-8	***P. calliantha***	**5/5**	**5/5**	**5/5**			
Pn-9	***P. fraudulenta***	**5/5**	**3/5**	**4/4**	**5**	**5**	**5**
Pn-10	***P. fraudulenta***				**5**	**5**	**4**
Pn-11	***P. fraudulenta***				**5**	**5**	**5**
Pn-12	***P. fraudulenta***	**5/5**	**5/4**	**5/5**	**5**	**5**	**5**
Pn-13	***P. calliantha***				**5**	**5**	**5**
Pn-15	***P. fraudulenta***				**5**	**5**	**5**
CLN47	***P. multiseries***	**5/5**	**5/5**	**5/5**	**5**	**5**	**5**

[a]replicates for the particulate fraction
[b]replicates for the dissolved fraction

Table 4: Morphometric summary of Pseudo-nitzschia cultures used in the present study.

Strain	Species	Length (µm)	Width (µm)	Central interspace	Poroids (in 1 µm)	Striae (in 10 µm)	Fibulae (in 10 µm)	Band striae (in 10 µm)	Rows of poroids
*	*P. multiseries*	55-169	2.8-5.3	Absent	4-7	9-16	9-16	19-30	3-4 (5)
Pn-1	*P. multiseries*	55-82 (66.3) {9.0} [6]	2.4-4 (3.2) {0.5} [8]	Absent	4-8 (5.7) {1.7} [4]	10-16 (11.9) {1.7} [10]	10-16 (12.5) {2.0} [8]	18-26 (21) {3.6} [4]	3-4
CLN47	*P. multiseries*	52-76 (64.0) {10.0} [4]	3.3-3.8 (3.6) {0.2} [4]	Absent					
*	*P. calliantha*	41-98	1.3-2.6	Present	4-6	34-39	15-22	42-48	1.0
Pn-2	*P. calliantha*	55-61 (57.8) {4.8} [5]	1.5-3.0 (2.4) {0.6} [6]	Present	6-7 (6.75) {0.5} [4]	32-40 (36) {5.6} [2]	10-11 (10.5) {0.7} [2]	40 (40) {0} [1]	1
Pn-3	*P. calliantha*	62-66 (64.5) {2.3} [4]	1.7-2.3 (2.0) {0.2} [5]	Present	4-6 (5.3) {1.2} [3]	34-42 (38) {5.7} [2]	15-22 (18.5) {5} [2]		1
Pn-4	*P. calliantha*	29-37 (31.7) {4.6} [3]	2-2.5 (2.2) {0.2} [4]	Present	5-6 (5.2) {0.7} [3]	35-38 (36.3) {1.2} [7]	16-19.2 (18) {1.1} [6]	34-49.6 (43.2) {8.2} [3]	1
Pn-6	*P. calliantha*	43-71 (56.1) {14.7} [4]	1.9-2.8 (2.3) {0.4} [5]	Present	5-6 (5.5) {0.7} [2]	39-40 (39.5) {0.7} [2]	20-22 (21) {1.4} [2]		1
Pn-7	*P. calliantha*	22-33 (26.7) {4.2} [5]	1.7-3.5 (2.5) {0.6} [6]	Present	4-6 (5) {1.4} [2]	32-34 (33) {1.4} [2]	12-13 (12.5) {0.7} [2]		1
Pn-8	*P. calliantha*	46-55 (48.7) {3.6} [6]	1.8-2.5 (2.0) {0.3} [4]	Present	5-6 (5) {0.4} [5]	36.6-40 (38.8) {1.4} [5]	17.1-21.7 (19.2) {2.0} [5]	37.7-40 (39.2) {1.3} [3]	1
Pn-13	*P. calliantha*	84-87 (85.3) {1.5} [3]	1.9-2.7 (2.3) {0.3} [7]	Present	4-5 (4.3) {0.6} [3]	24-27 (25.6) {1.1} [8]	10-14 (12.3) {1.3} [8]	30-35 (31.8) {2.4} [4]	1
Pn-14	*P. calliantha*	42-60 (50.7) {6.3} [5]	2-2.3 (2.1) {0.1} [3]	Present	5-6 (5.7) {0.6} [3]	34-42 (37.3) {4.2} [3]	20-24 (22) {2} [3]	44 (44) {0} [1]	1
Pn-16	*P. calliantha*	73-81 (77.7) {3.1} [6]	2.1-2.8 (2.4) {0.3} [5]	Present	4 (4) {0} [1]	32 (32) {0} [1]	17 (17) {0} [1]	38 (38) {0} [1]	1
Pn-17	*P. calliantha*	53-70 (58.4) {6.8} [9]	2.1-3.0 (2.5) {0.3} [9]	Present	4 (4) {0} [1]	36 (36) {0} [1]	19 (19) {0} [1]		1
Pn-18	*P. calliantha*	86.9 (86.9) {0} [1]	2.6 (2.6) {0} [1]	Present	4 (4) {0} [1]	35 (35) {0} [1]	17 (17) {0} [1]		1
*	*P. fraudulenta*	50-164	4.0-10	Present	4-7	17-26	12-26	35-40	2-3
Pn-9	*P. fraudulenta*	71-78 (74.4) {3.2} [5]	3-4 (3.8) {0.5} [4]	Present	5.6-7 (6.1) {0.8} [3]	21-24 (22.4) {1.0} [9]	18-20.6 (19.5) {0.8} [8]	33-40 (37.3) {2.6} [7]	2-3
Pn-10	*P. fraudulenta*	52-70 (60.0) {6.0} [6]	3-4 (3.4) {0.6} [3]	Present	6 (6) {0.6} [2]	20-24 (21.6) {2.0} [4]	16 (16) {0} [1]	36-40 (38) {2.0} [3]	2-3
Pn-11	*P. fraudulenta*	62-66 (64.3) {2.1} [3]	3-4 (3.7) {0.6} [3]	Present		20-23 (21.7) {1.2} [4]	20.8-22 (21.5) {0.6} [4]	36 (36) {0} [1]	2-3
Pn-12	*P. fraudulenta*	61-71 (64.5) {3.9} [5]	3.8-5.2 (4.6) {0.6} [4]	Present	6-6.4 (6.2) {0.3} [2]	20-24 (22.1) {2.0} [3]	22-24 (22.9) {1.0} [3]	28.8 (28.8) {0} [1]	2-3
Pn-15	*P. fraudulenta*	69-89 (80.2) {10.0} [4]	4.9-6 (5.6) {0.6} [3]	Present	5-6 (5.3) {0.6} [3]	20-23 (21.4) {1.1} [5]	11-20 (17) {3.5} [5]	32 (32) {0} [1]	2-3

The data are given as range of measurement (mean) {standard deviation} [replicates]. * Indicates measurements are referenced in other studies (Hasle 1965; Rivera 1985; Hasle et al. 1996; Skov et al. 1999; Stonik et al. 2001; Lundholm et al. 2003; Caroppo et al. 2005).

Table 5: Growth and toxicity of Pseudo-nitzschia cultures used in the present study.

Strain	Species	Salinity	Culture Volume (L)	Nitrogen Source	dissolved DA		particulate DA		growth μ (d^{-1})	Maximum Abundance
					(ng mL^{-1})	(fg $cell^{-1}$)	(ng mL^{-1})	(fg $cell^{-1}$)		
Pn-2	*P. calliantha*	15	1.5	NO_3^-	ND[a]	ND	ND	ND		
Pn-3	*P. calliantha*	15	1.5	NO_3^-	ND	ND	0.27	0.26	0.58	1.78 X 10^6
Pn-4	*P. calliantha*	15	1.5	NO_3^-	ND	ND	ND	ND	0.45	7.47 X 10^5
Pn-6	*P. calliantha*	15	1.5	NO_3^-	ND	ND	ND	ND	0.61	1.04 X 10^6
Pn-7	*P. calliantha*	15	1.5	NO_3^-	ND	ND	ND	ND	0.82	2.72 X 10^5
Pn-8	*P. calliantha*	15	1.5	NO_3^-	1.7	0.86	6.3	5.7	0.42	1.97 X 10^6
Pn-8	*P. calliantha*	15	0.065	NO_3^-	0.4 ± 0.49	2.0 ± 2.82	0.101 ± 0.0239	1.8 ± 2.51	0.55 ± 0.047	
Pn-8	*P. calliantha*	15	0.065	NH_4^+	0.3 ± 0.22	3.5 ± 5.41	0.14 ± 0.050	4.7 ± 7.7	0.87 ± 0.154	
Pn-8	*P. calliantha*	15	0.065	urea	1.2 ± 2.67	19.8 ± 44.26	0.17 ± 0.057	3.5 ±2.66	0.42 ± 0.056	
Pn-13	*P. calliantha*	15	1.5	NO_3^-	+[b]	+	+	+	0.68	3.64 X 10^5
Pn-13	*P. calliantha*	15	0.065	NO_3^-					0.72 ± 0.071	
Pn-13	*P. calliantha*	15	0.065	NH_4^+					0.71 ± 0.064	
Pn-13	*P. calliantha*	15	0.065	urea					0.44 ± 0.028	
Pn-16	*P. calliantha*	15	1.5	NO_3^-	ND	ND	ND	ND	0.9	1.41 X 10^5
Pn-17	*P. calliantha*	15	1.5	NO_3^-	ND	ND	ND	ND	0.9	4.61 X 10^5
Pn-9	*P. fraudulenta*	32	1.5	NO_3^-	ND	ND		0.667	0.56	7.33 X 10^5
Pn-9	*P. fraudulenta*	32	0.065	NO_3^-	0.6 ± 0.71	32 ± 43.3	0.009 ± 0.011	0.29 ± 0.389	0.75 ± 0.051	
Pn-9	*P. fraudulenta*	32	0.065	NH_4^+	0.9 ± 0.55	20 ± 11.0	ND	ND	0.62 ± 0.038	
Pn-9	*P. fraudulenta*	32	0.065	urea	0.2 ± 0.21	4.7 ± 5.95	ND	ND	1.02 ± 0.137	
Pn-10	*P. fraudulenta*	32	1.5	NO_3^-	ND	ND	ND	ND	0.6	2.49 X 10^5
Pn-10	*P. fraudulenta*	32	0.065	NO_3^-					0.84 ± 0.035	
Pn-10	*P. fraudulenta*	32	0.065	NH_4^+					0.85 ± 0.058	
Pn-10	*P. fraudulenta*	32	0.065	urea					0.58 ± 0.067	

Table 5 cont.

Strain	Species	Salinity	Culture Volume (L)	Nitrogen Source	dissolved DA		particulate DA		growth μ (d^{-1})	Maximum Abundance
					(ng mL^{-1})	(fg $cell^{-1}$)	(ng mL^{-1})	(fg $cell^{-1}$)		
Pn-11	*P. fraudulenta*	32	1.5	NO_3^-	ND	ND	ND	ND	0.46	2.18×10^5
Pn-11	*P. fraudulenta*	32	0.065	NO_3^-					0.73 ± 0.036	
Pn-11	*P. fraudulenta*	32	0.065	NH_4^+					1.22 ± 0.177	
Pn-11	*P. fraudulenta*	32	0.065	urea					0.55 ± 0.103	
Pn-12	*P. fraudulenta*	32	1.5	NO_3^-	0.525	4.6	0.057	1.6	0.37	1.59×10^5
Pn-12	*P. fraudulenta*	32	0.065	NO_3^-	0.06 ± 0.139	2.7 ± 5.92	0.015 ± 0.0092	0.42 ± 0.257	0.75 ± 0.039	
Pn-12	*P. fraudulenta*	32	0.065	NH_4^+	0.8 ± 0.38	30 ± 17.0	ND	ND	0.88 ± 0.048	
Pn-12	*P. fraudulenta*	32	0.065	urea	1.2 ± 0.24	53 ± 19.7	0.019 ± 0.0269	0.8 ± 1.03	0.58 ± 0.181	
Pn-15	*P. fraudulenta*	32	1.5	NO_3^-	ND	ND	ND	ND	1.2	2.27×10^5
Pn-15	*P. fraudulenta*	32	0.065	NO_3^-					1.05 ± 0.033	
Pn-15	*P. fraudulenta*	32	0.065	NH_4^+					1.16 ± 0.020	
Pn-15	*P. fraudulenta*	32	0.065	urea					1.05 ± 0.030	
CLN47	*P. multiseries*	32	1.5	NO_3^-	3.76	57.2	69.6	1059	0.8	1.14×10^5
CLN47	*P. multiseries*	32	0.065	NO_3^-	1.2 ± 0.33	8.9 ± 2.98	48 ± 29.1	362 ± 254.3	0.65 ± 0.018	
CLN47	*P. multiseries*	32	0.065	NH_4^+	0.19 ± 0.039	1.8 ± 0.35	5.0 ± 2.77	48 ± 23.6	0.61 ± 0.054	
CLN47	*P. multiseries*	32	0.065	urea	0.16 ± 0.094	1.0 ± 0.54	3.9 ± 5.53	24 ± 32.6	0.68 ± 0.070	
Pn-1	*P. multiseries*	15	1.5	NO_3^-	17.3	20	398	458	0.45	1.14×10^6
Pn-1	*P. multiseries*	15	0.065	NO_3^-	30 ± 3.7	584 ± 169.4	65 ± 11.2	1250 ± 351.8	0.76 ± 0.104	
Pn-1	*P. multiseries*	15	0.065	NH_4^+	103 ± 11.5	2134 ± 154.2	87 ± 25.1	406 ± 53.7	0.76 ± 0.175	
Pn-1	*P. multiseries*	15	0.065	urea	2.9 ± 1.10	61 ± 16.8	19 ± 3.5	1807 ± 514.3	0.30 ± 0.100	

[a]ND indicates that DA was not found.
[b]+ indicates that toxin was found, but not quantified.

Table 6: Nested ANOVA tables for growth and toxicity experiments using different nitrogen sources.

Growth (Fig. 5)	df	SS	MS	F	P
nitrogen	2	1.22	0.611	77.1	<0.001
species nested in nitrogen	6	1.79	0.298	37.6	<0.001
strain nested in species nested in nitrogen	18	3.39	0.188	23.7	<0.001
replicate cultures	106	0.84	0.008		

Dissolved DA cell^{-1} (Fig. 7)	df	SS	MS	F	P
nitrogen	2	1751435	875717	216.7	<0.001
species nested in nitrogen	6	6875495	1145916	283.5	<0.001
strain nested in species nested in nitrogen	6	12139455	2023243	500.6	<0.001
replicate cultures	56	226342	4042		

Dissolved DA mL^{-1} (Fig. 6)	df	SS	MS	F	P
nitrogen	2	4038	2019	180.5	<0.001
species nested in nitrogen	6	16393	2732	244.3	<0.001
strain nested in species nested in nitrogen	6	28338	4723	422.3	<0.001
replicate cultures	56	626	11		

Particulate DA cell^{-1} (Fig. 7)	df	SS	MS	F	P
nitrogen	2	935125	467562	13.8	<0.001
species nested in nitrogen	6	8974923	1495821	44.3	<0.001
strain nested in species nested in nitrogen	6	10097263	1682877	49.8	<0.001
replicate cultures	58	1958029	33759		

Particulate DA mL^{-1} (Fig. 6)	df	SS	MS	F	P
nitrogen	2	3522	1761	15.5	<0.001
species nested in nitrogen	6	31698	5283	46.5	<0.001
strain nested in species nested in nitrogen	6	18149	3025	26.6	<0.001
replicate cultures	58	6595	114		

Total DA cell^{-1} (Fig. 7)	df	SS	MS	F	P
nitrogen	2	136266	68133	1.7	0.200
species nested in nitrogen	6	21394034	3565672	87.8	<0.001
strain nested in species nested in nitrogen	6	28286001	4714333	116.1	<0.001
replicate cultures	60	2436325	40605		

Total DA mL^{-1} (Fig. 6)	df	SS	MS	F	P
nitrogen	2	11648	5824	67.3	<0.001
species nested in nitrogen	6	85654	14276	165.0	<0.001
strain nested in species nested in nitrogen	6	90050	15008	173.5	<0.001
replicate cultures	56	4845	87		

Table 7: Results of ANOVA and Tukey's studentized range multiple comparisons test ($\alpha = 0.05$) for domoic acid content (normalized to culture volume and cell number) in Pseudo-nitzschia strains grown on NO_3^-, NH_4^+ and urea. Pn-1 and CLN47 are P. multiseries. Pn-8 is P. calliantha. Pn-9 and Pn-12 are P. fraudulenta.

Strain	per mL									per cell								
	particulate			dissolved			total			particulate			dissolved			total		
	NH_4^+	NO_3^-	urea	NH_4^+	NO_3^-	urea	NH_4^+	NO_3^-	urea	NH_4^+	NO_3^-	urea	NH_4^+	NO_3^-	urea	NH_4^+	NO_3^-	urea
Pn-1	A*	A*	B	A*	B	C	A*	B	C	A*	A*	B	A*	B	C	A*	B	C
Pn-8	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Pn-9	A	A	A	A	A	A	A	A	A	A	B*	A	A	A	A	A	A	A
Pn-12	A*	B	A*	A	A	A	A*	B	A*	A	A	A	AB	B	A*	A	A	A
CLN47	AB	A*	B	AB	A*	B	AB	A*	B	B	A*	B	B	A*	B	B	A*	B

Data (in Figures 6 and 7) for the particulate fraction, dissolved fraction and total DA (dissolved + particulate) content were tested separately. Treatments with different letters indicate significantly different means. *Indicate treatments with higher toxin content.

Table 8**:** Spearman's rank coefficient (ρ) for growth rate (μ) and DA content. Data were grouped and analyzed by strain.

Strain	Particulate DA (ng mL^{-1})		Dissolved DA (ng mL^{-1})		Total DA (ng mL^{-1})		Particulate DA (fg $cell^{-1}$)		Dissolved DA (fg $cell^{-1}$)		Total DA (fg $cell^{-1}$)	
	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value
CLN47	**-0.1210**	**0.6569**	**-0.1430**	**0.6006**	**-0.1210**	**0.6569**	**-0.1210**	**0.6569**	**-0.2332**	**0.396**	**-0.1210**	**0.6569**
Pn-1	**0.7377***	**0.0059**	**0.6571***	**0.0142**	**0.6893***	**0.0101**	**0.6893***	**0.0101**	**0.6231***	**0.0201**	**0.6643***	**0.0132**
Pn-8	**-0.4554**	**0.0989**	**0.0916**	**0.7471**	**0.1826**	**0.5154**	**-0.2860**	**0.2987**	**0.2138**	**0.4455**	**-0.1293**	**0.6474**
Pn-9	**-0.0974**	**0.7196**	**-0.5479***	**0.0473**	**-0.5385**	**0.0513**	**-0.1046**	**0.7002**	**-0.5868***	**0.0337**	**-0.5868***	**0.0337**
Pn-12	**-0.2426**	**0.3953**	**-0.0530**	**0.8469**	**-0.0578**	**0.8339**	**-0.1944**	**0.4947**	**-0.1757**	**0.5364**	**-0.1788**	**0.5294**

*Indicates significance at 0.05 level

Table 9: Growth vs. irradiance curve parameters for three strains of two species of Pseudo-nitzschia grown in three temperatures. Pn-1 and CLN47 are P. multiseries. Pn-15 is P. fraudulenta.

Strain	temp (°C)	α (d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$)	β (d^{-1}[µmol photons m^{-2} sec^{-1}]$^{-1}$)	μ_{max} (d^{-1})	E_k (µmol photons m^{-2} sec^{-1})
Pn-1	**10**	**0.0067 (0.0016)^{A}**	**2.4X10^{-4} (0.0002)**	**0.24 (0.045)**	**36 (15)**
	15	**0.0047 (0.0016)^{C}**	**0**	**0.32 (0.148)**	**70 (55)**
	20	**0.0098 (0.0026)**	**0.003 (0.0107)**	**2.0 (3.4)**	**200 (410)**
CLN47	**10**	**0.0127 (0.0030)^{B}**	**2X10^{-4} (0.00047)**	**0.33 (0.071)**	**26 (12)**
	15	**0.0109 (0.0025)**	**0**	**0.33 (0.057)^{A}**	**30 (12.0)**
	20	**0.0094 (0.0052)**	**0**	**?**	**?**
Pn-15	**10**	**0.0263 (0.0001)^{AB}**	**0**	**?**	**?**
	15	**0.0239 (0.0047)^{C}**	**0.0004 (0.0003)**	**0.68 (0.072)^{A}**	**28 (9)**
	20	**?**	**?**	**0.78 (0.04)**	**?**

Values for α, β, μ_{max} and E_k are given with standard error in parentheses. Identical letter superscripts indicate significant difference between parameters at the 0.05 level. Photoinhibition (β) was not tested statistically due to missing values. Data are in Figure 10.

Table 10: Age of cultures in years at the time of experimentation.

Strain	Species	Culture Age (year)		
		Toxin[a]	Nitrogen[b]	Irradiance[c]
Pn-1	***P. multiseries***	**1.5**	**2.5**	**2.2**
Pn-3	***P. calliantha***	**1**		
Pn-4	***P. calliantha***	**0.3**		
Pn-6	***P. calliantha***	**0.3**		
Pn-7	***P. calliantha***	**0.5**		
Pn-8	***P. calliantha***	**0.5**	**1.5**	
Pn-9	***P. fraudulenta***	**0.25**	**1.2**	
Pn-10	***P. fraudulenta***	**0.75**	**1.2**	
Pn-11	***P. fraudulenta***	**0.75**	**1.2**	
Pn-12	***P. fraudulenta***	**0.75**	**1.3**	
Pn-13	***P. calliantha***	**0.6**	**1**	
Pn-15	***P. fraudulenta***	**0.25**	**0.2**	**0.5**
CLN47	***P. multiseries***	**1.2**	**1.3**	**1.5**
Pn-16	***P. calliantha***	**0.2**		
Pn-17	***P.calliantha***	**0.2**		

[a]Results in Figures 3 and 4
[b]Results in Figures 5, 6 and 7
[c]Results in Figure 10.

Figure 1: TEM micrographs of digested frustules of Pseudo-nitzschia cultures. A, B and C (scale bar = 1 μm) show arrangement of poroids. D, E and F (scale bar = 5μm) show presence or absence of central interspace (arrows) and length of transapical axis. A and F are P. fraudulenta (Pn-12), B and E are P. calliantha (Pn-13) and C and D are P. multiseries (Pn-1).

Figure 2: Parsimony tree inferred for the D1-D3 variable region of LSU (A; 720 positions included) and for the ITS1-5.8S-ITS2 region (B; approx. 990 positions included) from Chesapeake Bay isolates of Pseudo-nitzschia (in bold) and sequence data available on GenBank. Analysis was performed using heuristic searches (25X random addition of sequences) with TBR (tree bisection and reconnection) branch swapping. Gaps were treated as a fifth character state in figure 2A, and the approximate number of character changes are shown on branches. Each species includes culture identification, location and GenBank accession number.

Figure 3: Abundance (open squares) and DA concentration normalized to culture volume (diamonds) over time in six Pseudo-nitzschia strains of three species grown in silicate-limited medium with NO_3^- as a nitrogen source. DA concentrations represent the sum of particulate and dissolved fractions. Open diamonds represent semi-quantitative data (<LOQ, but >LOD). Closed diamonds represent quantitative values (>LOQ). Numbers shown within diamonds indicate LOD and LOQ for each sample in pg mL^{-1} which can be found in Table 2. Zero values were plotted as values of one. (A) Pn-1, P. multiseries. (B) CLN47, P. multiseries. (C) Pn-8, P. calliantha. (D) Pn-3, P. calliantha. (E) Pn-12, P. fraudulenta. (F) Pn-9, P. fraudulenta.

Figure 4: Abundance (open squares) and DA concentration normalized to cell number (diamonds) over time in six Pseudo-nitzschia strains of three species. DA concentrations represent the sum of particulate and dissolved fractions. Closed diamonds represent quantitative values (definitions as in Fig. 3). LOD and LOQ for each sample are given in Table 2. Zero values were plotted as values of 1. (A) Pn-1, P. multiseries. (B) CLN47, P. multiseries. Open diamonds indicate samples where dissolved fraction was below LOQ. (C) Pn-8, P. calliantha. (D) Pn-3, P. calliantha. (E) Pn-12, P. fraudulenta. (F) Pn-9, P. fraudulenta.

Figure 5: Specific growth rates (μ) for strains of Pseudo-nitzschia grown on NO_3^-, NH_4^+ and urea. Error bars represent one SD. Means with identical letters above bars are not significantly different at 0.05 using Tukey's studentized range test for each strain. Strain designations are given along the x axis and species names are given at the top of the figure. Replication is shown in Table 3.

Figure 6: DA content (ng mL^{-1}) one week after fluorescence peak in strains of Pseudo-nitzschia grown on NO_3^-, NH_4^+ and urea in silicate-limited media. Filled bars show particulate DA and open bars show dissolved DA. Error bars represent one SD. Letters showing significant difference are in Table 7. Replication is shown in Table 3. Note the differences in scale. (A) Pn-1, P. multiseries (B) CLN47, P. multiseries. Mean dissolved DA in NH_4^+ and urea treatments contain semi-quantitative data (<LOQ, but >LOD). (C) Pn-8, P. calliantha. Mean dissolved DA in the NH_4^+ treatment contains semi-quantitative data. (D) Pn-12, P. fraudulenta. Mean dissolved DA in the NH_4^+ and NO_3^- treatments and particulate DA in the NO_3^- and urea treatments contain semi-quantitative data. (E) Pn-9, P. fraudulenta. Mean dissolved DA in all treatments and particulate DA in the NO_3^- treatment contain semi-quantitative data.

Figure 7: DA content (fg $cell^{-1}$) one week after fluorescence peak for strains of Pseudo-nitzschia grown on NO_3^-, NH_4^+ and urea in silicate limited media. Filled bars show particulate DA and open bars show dissolved DA. Error bars represent one SD. Letters showing significant difference are in Table 7. Replication is shown in Table 3. Note the difference in scale. (A) Pn-1, P. multiseries (B) CLN47, P. multiseries. Mean dissolved DA in NH_4^+ and urea treatments contain semi-quantitative data (<LOQ, but >LOD). (C) Pn-8, P. calliantha. Mean dissolved DA in the NH_4^+ treatment contains semi-quantitative data. (D) Pn-12, P. fraudulenta. Mean dissolved DA in the NH_4^+ and NO_3^- treatments and particulate DA in the NO_3^- and urea treatments contain semi-quantitative data. (E) Pn-9, P. fraudulenta. Mean dissolved DA in all treatments and particulate DA in the NO_3^- treatment contain semi-quantitative data.

Figure 8: Growth rates in exponential phase (shaded bars) and total DA content (ng mL^{-1}; open bars) one week after fluorescence peak in strains of Pseudo-nitzschia grown in batch culture on NO_3^-, NH_4^+ and urea in silicate-limited media. Error bars represent one SD. Spearman's correlation coefficients are in Table 8. Replication is shown in Table 3. Note the difference in scale. (A) Pn-1, P. multiseries (B) CLN47, P. multiseries. (C) Pn-8, P. calliantha. (D) Pn-12, P. fraudulenta. (E) Pn-9, P. fraudulenta.

Figure 9: Growth rates in exponential phase (shaded bars) and total DA content (fg $cell^{-1}$; open bars) one week after fluorescence peak in strains of Pseudo-nitzschia grown in batch culture on NO_3^-, NH_4^+ and urea in silicate-limited media. Error bars represent one SD. Spearman's correlation coefficients are in Table 8. Replication is shown in Table 3. Note the difference in scale. (A) Pn-1, P. multiseries (B) CLN47, P. multiseries. (C) Pn-8, P. calliantha. (D) Pn-12, P. fraudulenta. (E) Pn-9, P. fraudulenta.

Figure 10: Growth vs. irradiance curves for three strains of two species of Pseudo-nitzschia at three temperatures (10°, 15°, 20°C) in nutrient replete medium. (A) Pn-1, P. multiseries (B) CLN47, P. multiseries (C) Pn-15, P. fraudulenta. Photosynthetic parameters derived from curves are summarized in Table 9.

+ - + -

+ - + -

Fig. 1

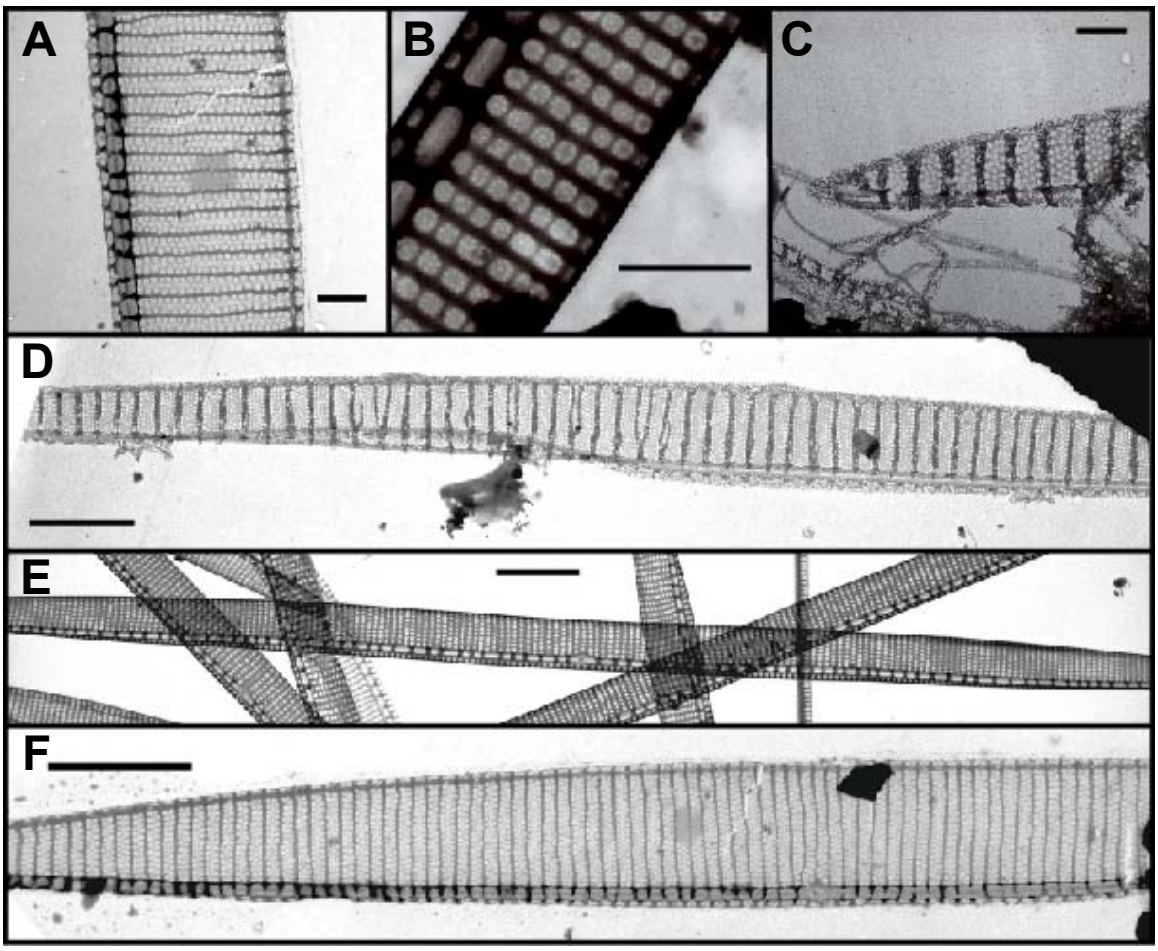
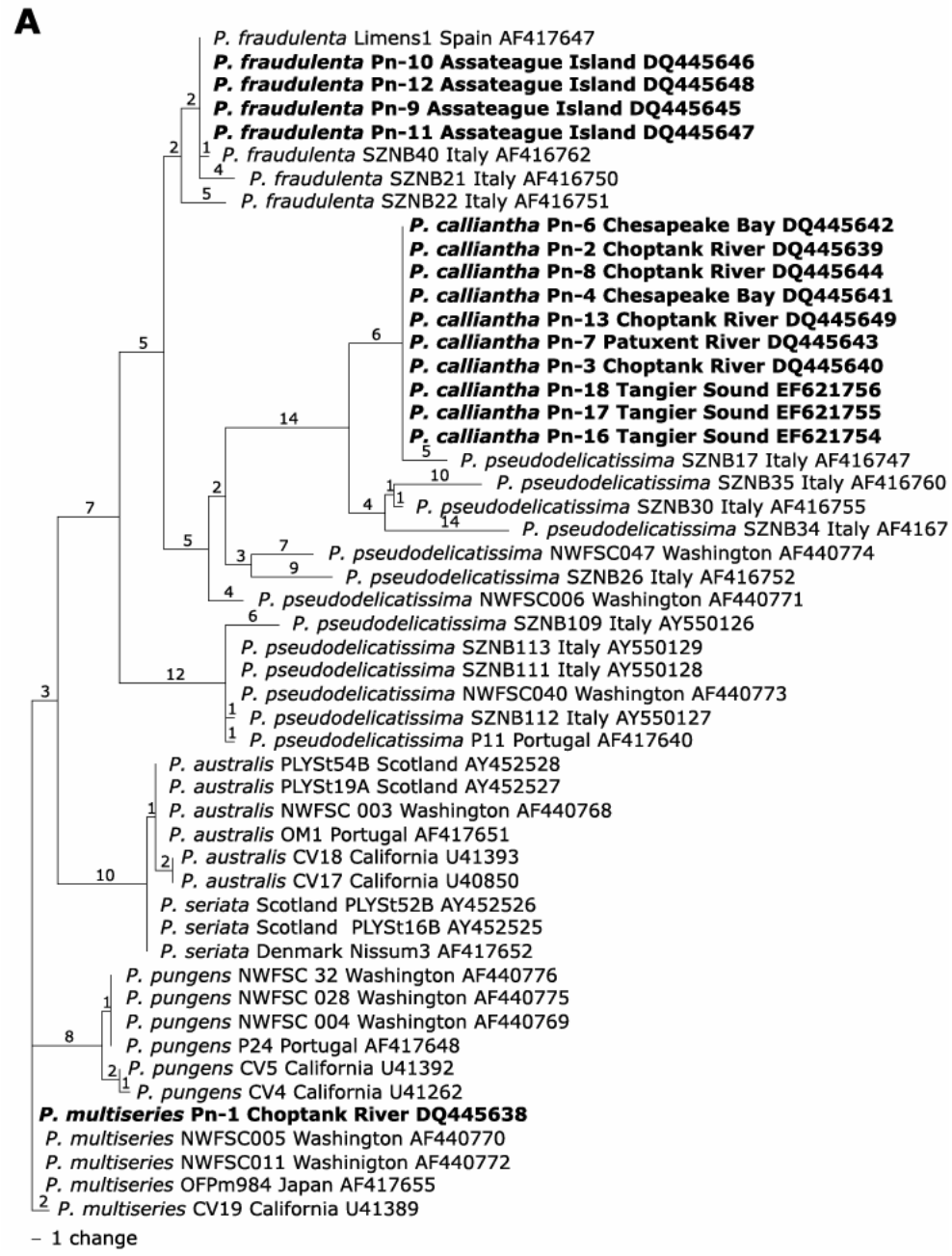


Fig. 2A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P. fraudulenta Pn-10 Assateague Island DQ445646
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P. fraudulenta Pn-11 Assateague Island DQ445647
P. fraudulenta SZNB40 Italy AF416762
P. fraudulenta SZNB21 Italy AF416750
P. fraudulenta SZNB22 Italy AF416751
P. calliantha Pn-6 Chesapeake Bay DQ445642
P. calliantha Pn-2 Choptank River DQ445639
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P. calliantha Pn-13 Choptank River DQ445649
P. calliantha Pn-7 Patuxent River DQ445643
P. calliantha Pn-3 Choptank River DQ445640
P. calliantha Pn-18 Tangier Sound EF621756
P. calliantha Pn-17 Tangier Sound EF621755
P. calliantha Pn-16 Tangier Sound EF621754
P. pseudodelicatissima SZNB17 Italy AF416747
P. pseudodelicatissima SZNB35 Italy AF416760
P. pseudodelicatissima SZNB30 Italy AF416755
P. pseudodelicatissima SZNB34 Italy AF4167
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P. pseudodelicatissima SZNB26 Italy AF416752
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P. pungens NWFSC 32 Washington AF440776
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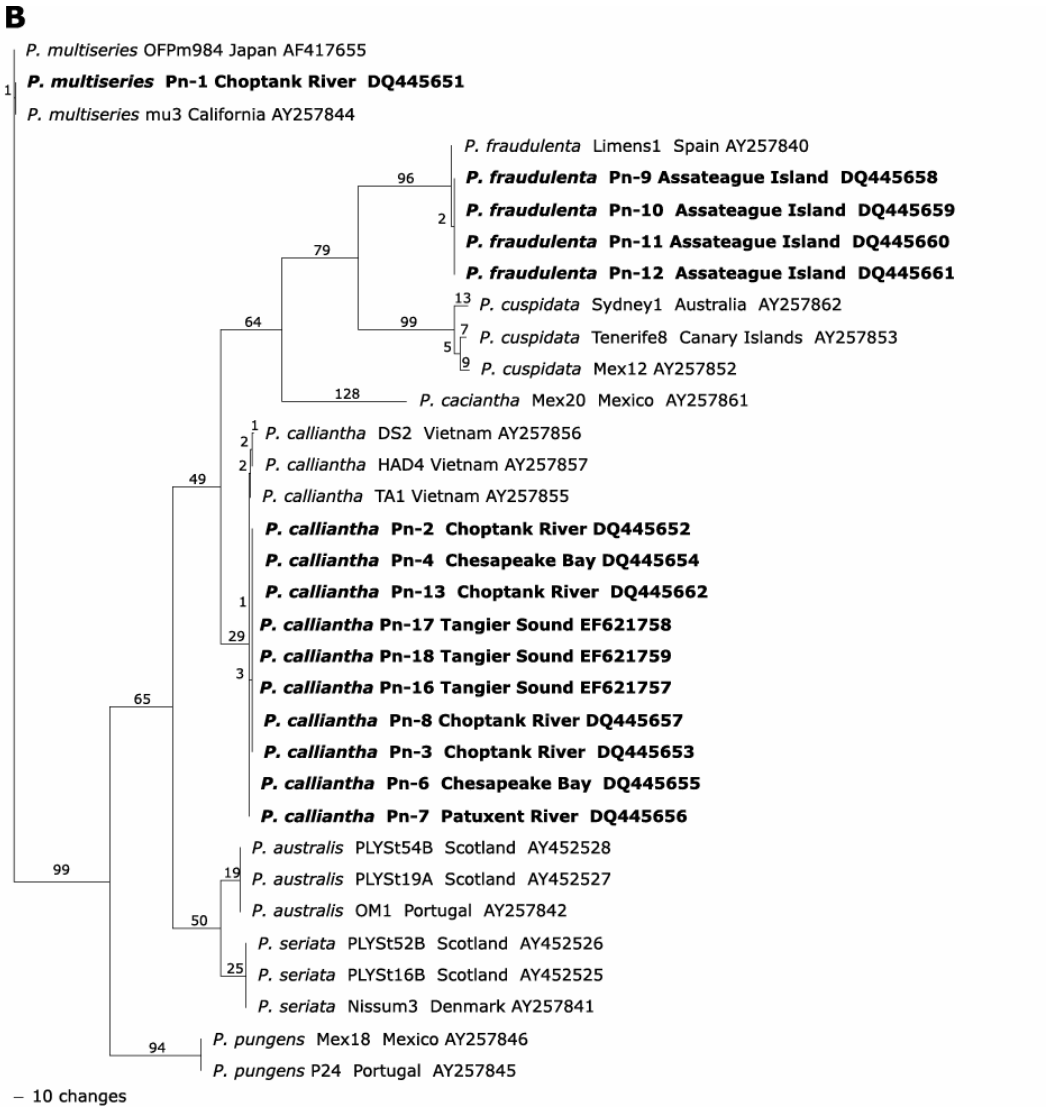
Fig. 2B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P. fraudulenta Limens1 Spain AY257840
P. fraudulenta Pn-9 Assateague Island DQ445658
P. fraudulenta Pn-10 Assateague Island DQ445659
P. fraudulenta Pn-11 Assateague Island DQ445660
P. fraudulenta Pn-12 Assateague Island DQ445661
P. cuspidata Sydney1 Australia AY257862
P. cuspidata Tenerife8 Canary Islands AY257853
P. cuspidata Mex12 AY257852
P. caciantha Mex20 Mexico AY257861
P. calliantha DS2 Vietnam AY257856
P. calliantha HAD4 Vietnam AY257857
P. calliantha TA1 Vietnam AY257855
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P. calliantha Pn-13 Choptank River DQ445662
P. calliantha Pn-17 Tangier Sound EF621758
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P. calliantha Pn-16 Tangier Sound EF621757
P. calliantha Pn-8 Choptank River DQ445657
P. calliantha Pn-3 Choptank River DQ445653
P. calliantha Pn-6 Chesapeake Bay DQ445655
P. calliantha Pn-7 Patuxent River DQ445656
P. australis PLYSt54B Scotland AY452528
P. australis PLYSt19A Scotland AY452527
P. australis OM1 Portugal AY257842
P. seriata PLYSt52B Scotland AY452526
P. seriata PLYSt16B Scotland AY452525
P. seriata Nissum3 Denmark AY257841
P. pungens Mex18 Mexico AY257846
P. pungens P24 Portugal AY257845
10 changes

Fig. 3

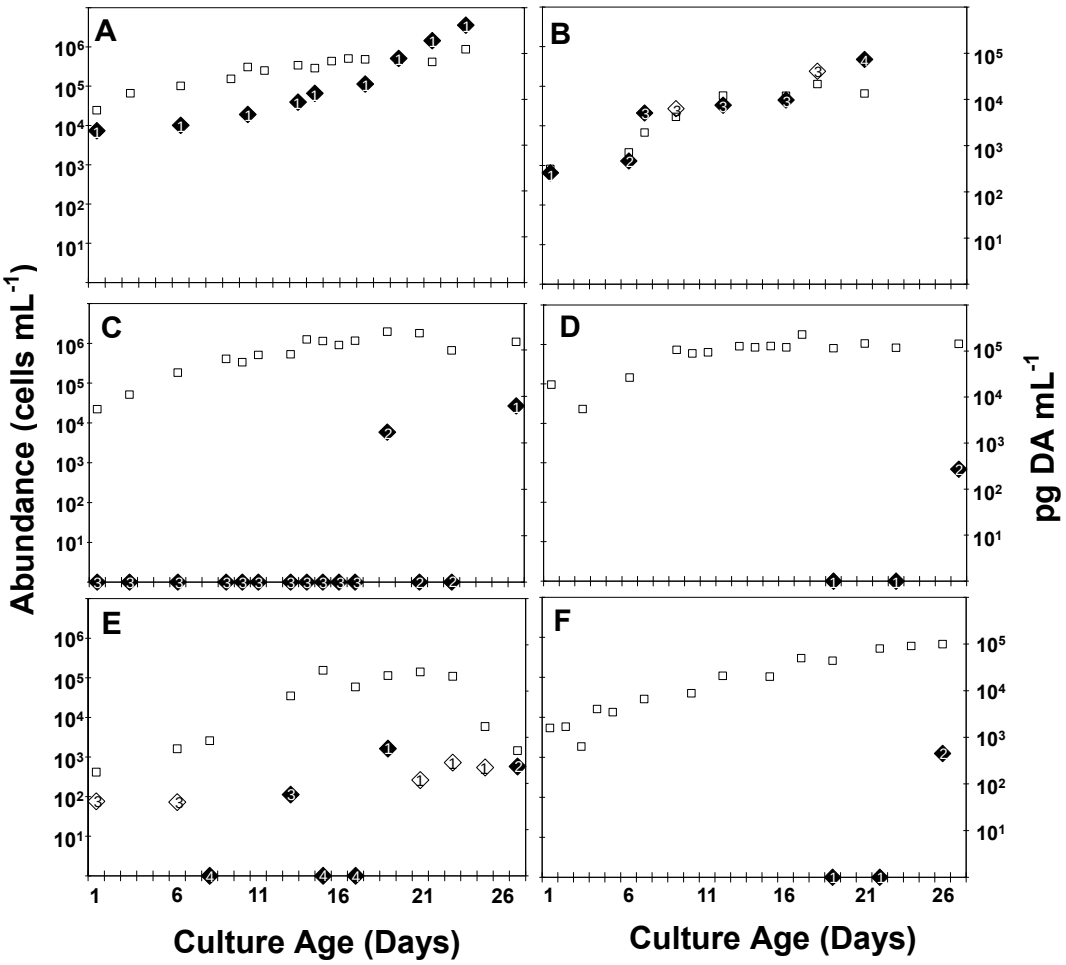

A
B
C
D
E
F
Abundance (cells mL-1)
pg DA mL-1
Culture Age (Days)
Culture Age (Days)
1
6
11
16
21
26

Fig. 4

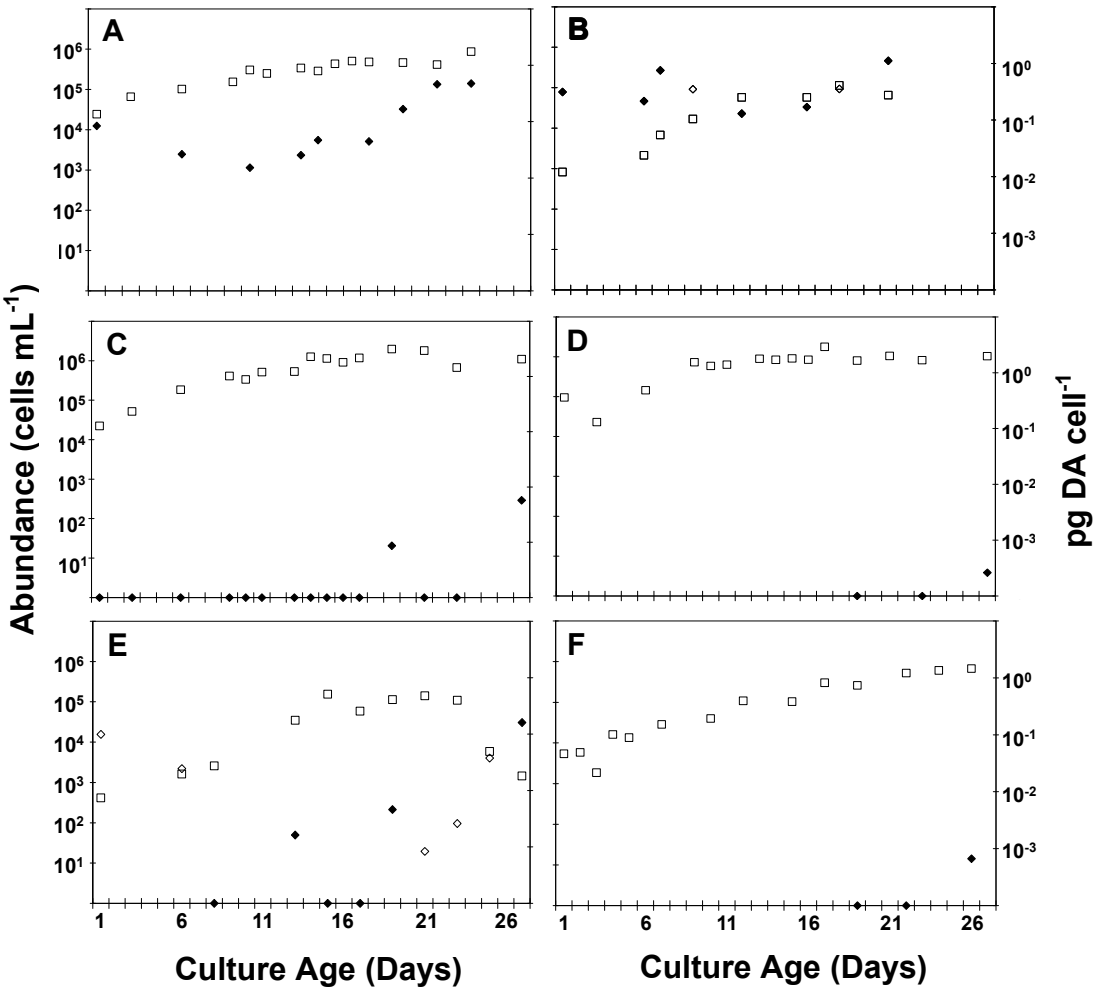

A
B
C
D
E
F
Abundance (cells mL-1)
pg DA cell-1
Culture Age (Days)
Culture Age (Days)
1
6
11
16
21
26

Fig. 5

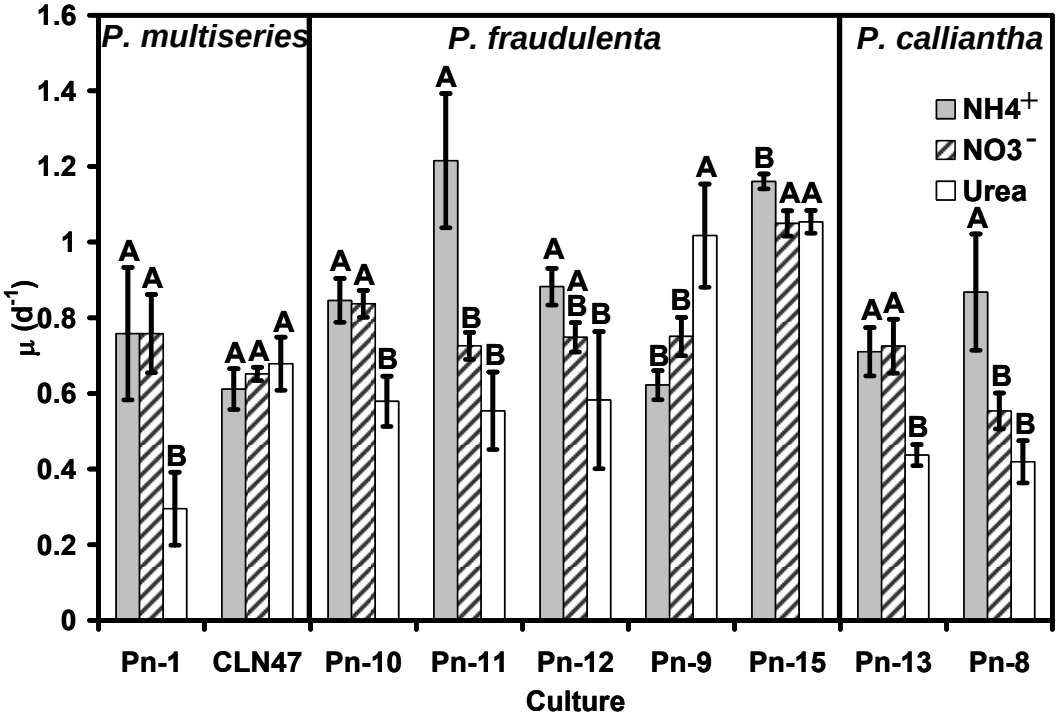

P. multiseries
P. fraudulenta
P. calliantha
NH4+
NO3-
Urea
μ (d-1)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pn-1
CLN47
Pn-10
Pn-11
Pn-12
Pn-9
Pn-15
Pn-13
Pn-8
Culture

Fig. 6

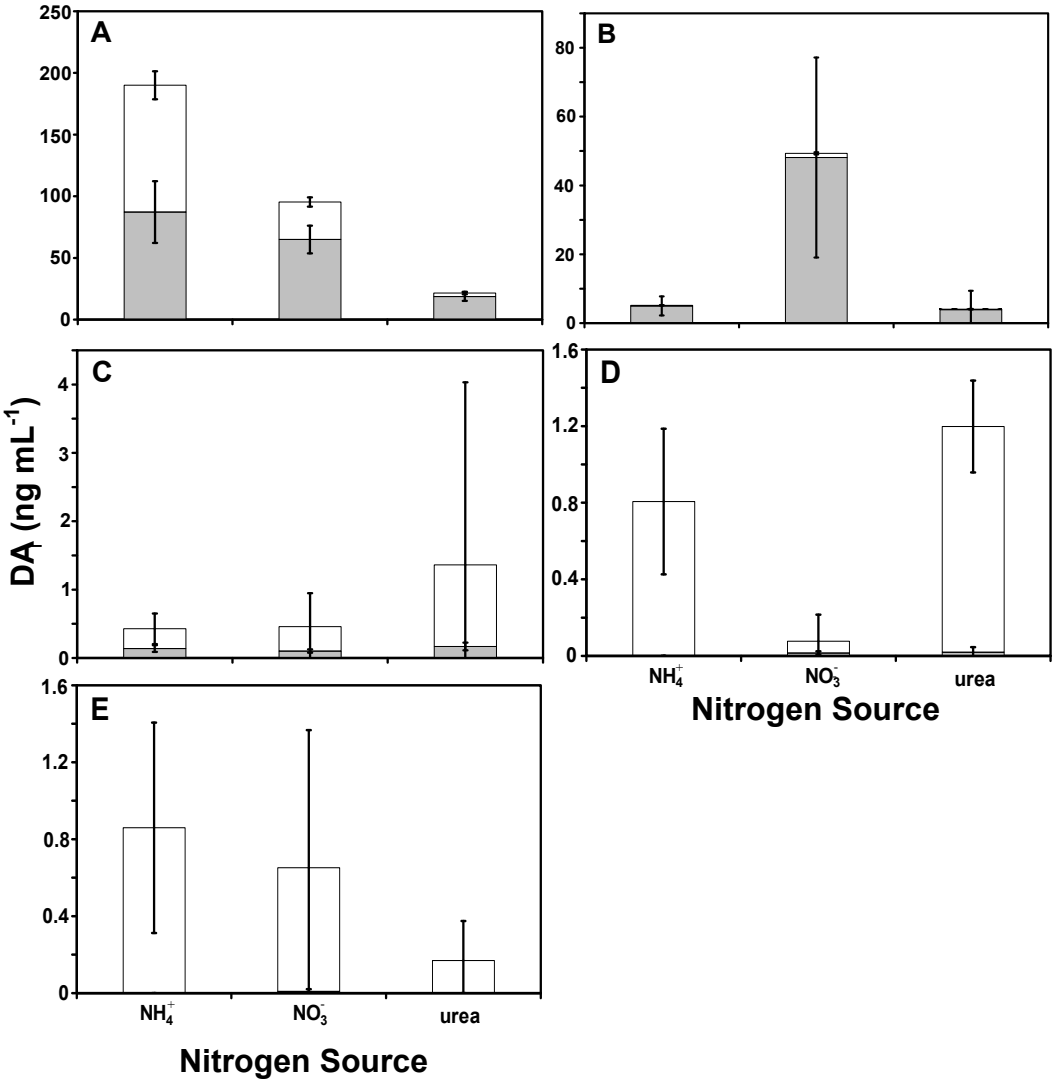


Fig. 7

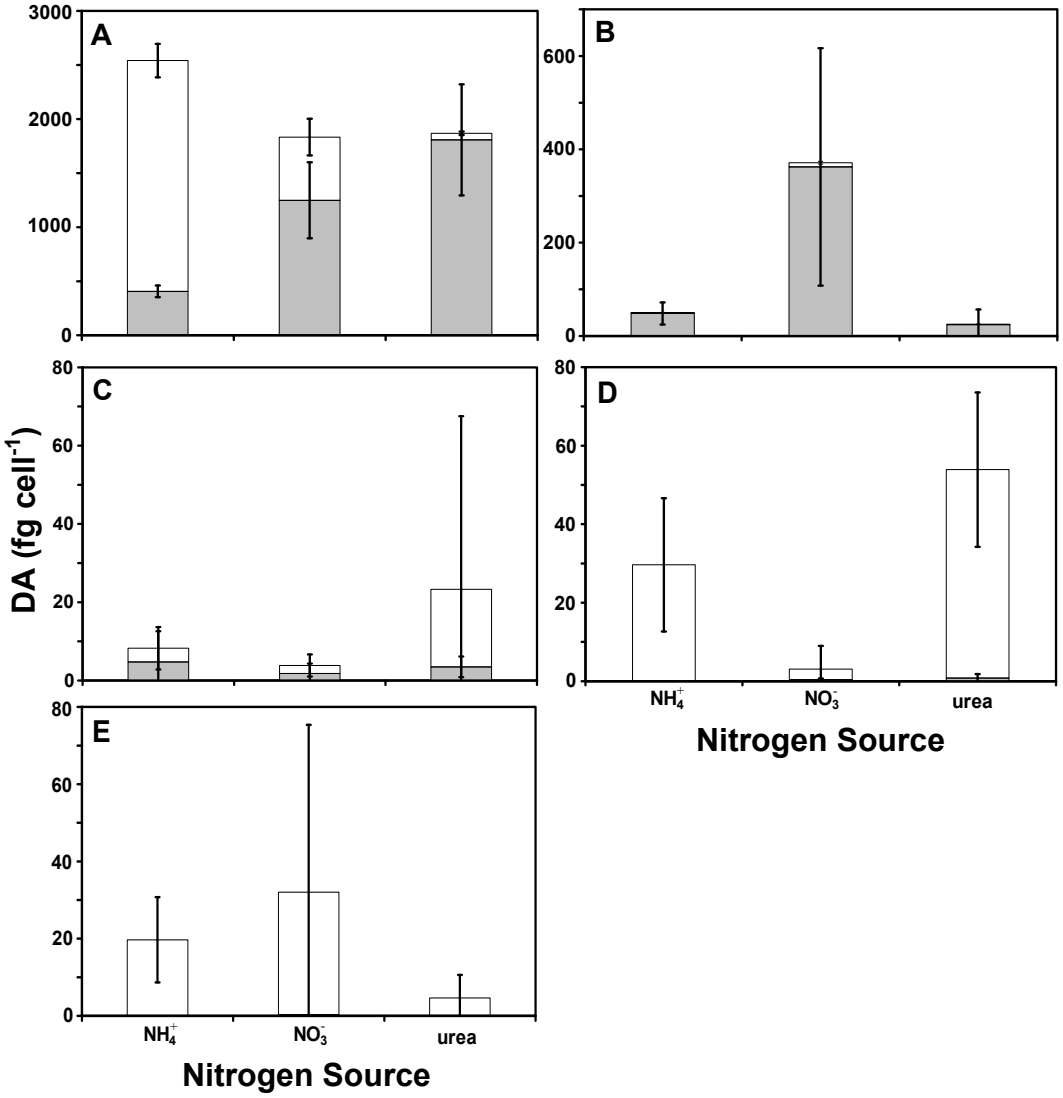


Fig. 8

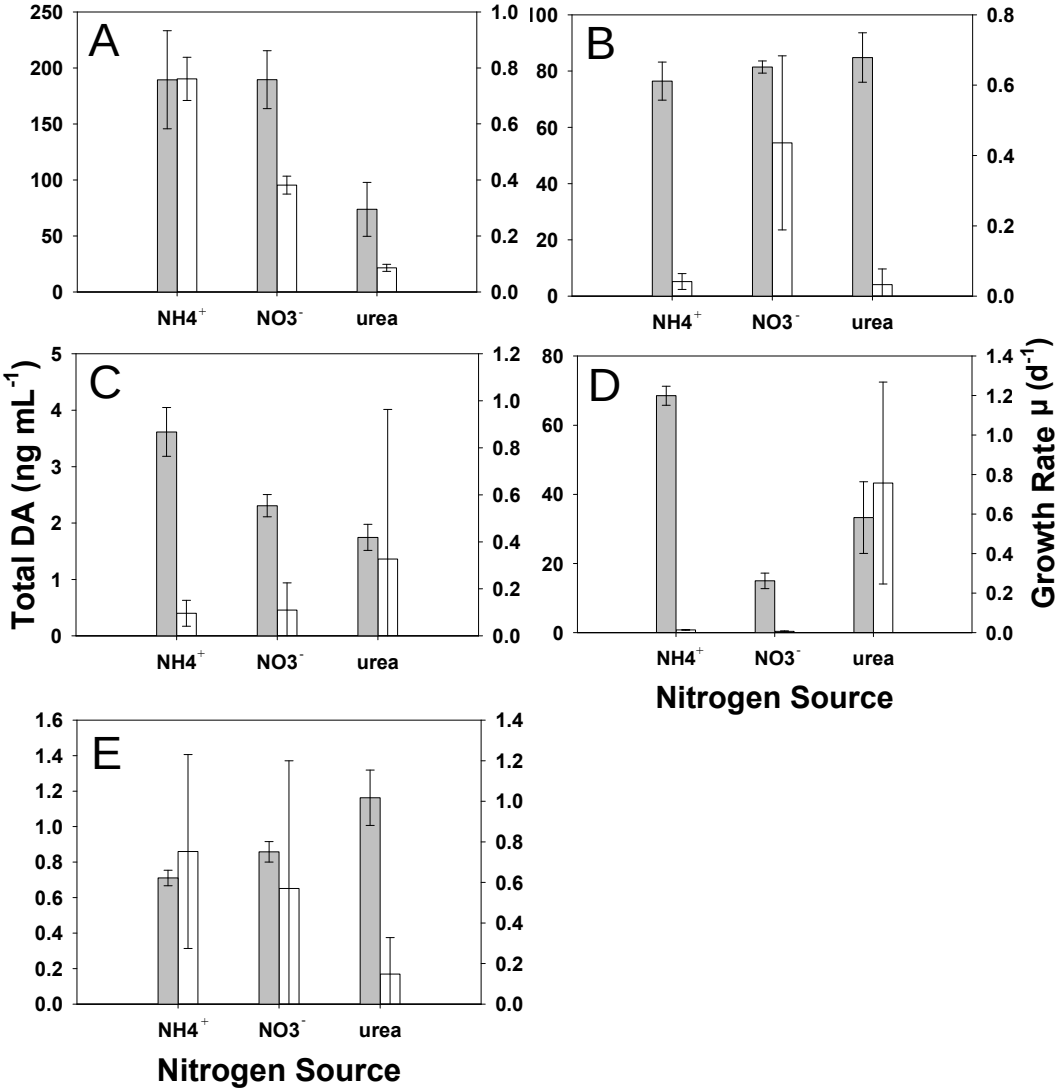

A
B
C
D
E
Total DA (ng mL-1)
Growth Rate μ (d-1)
NH4+
NO3-
urea
Nitrogen Source

Fig. 9

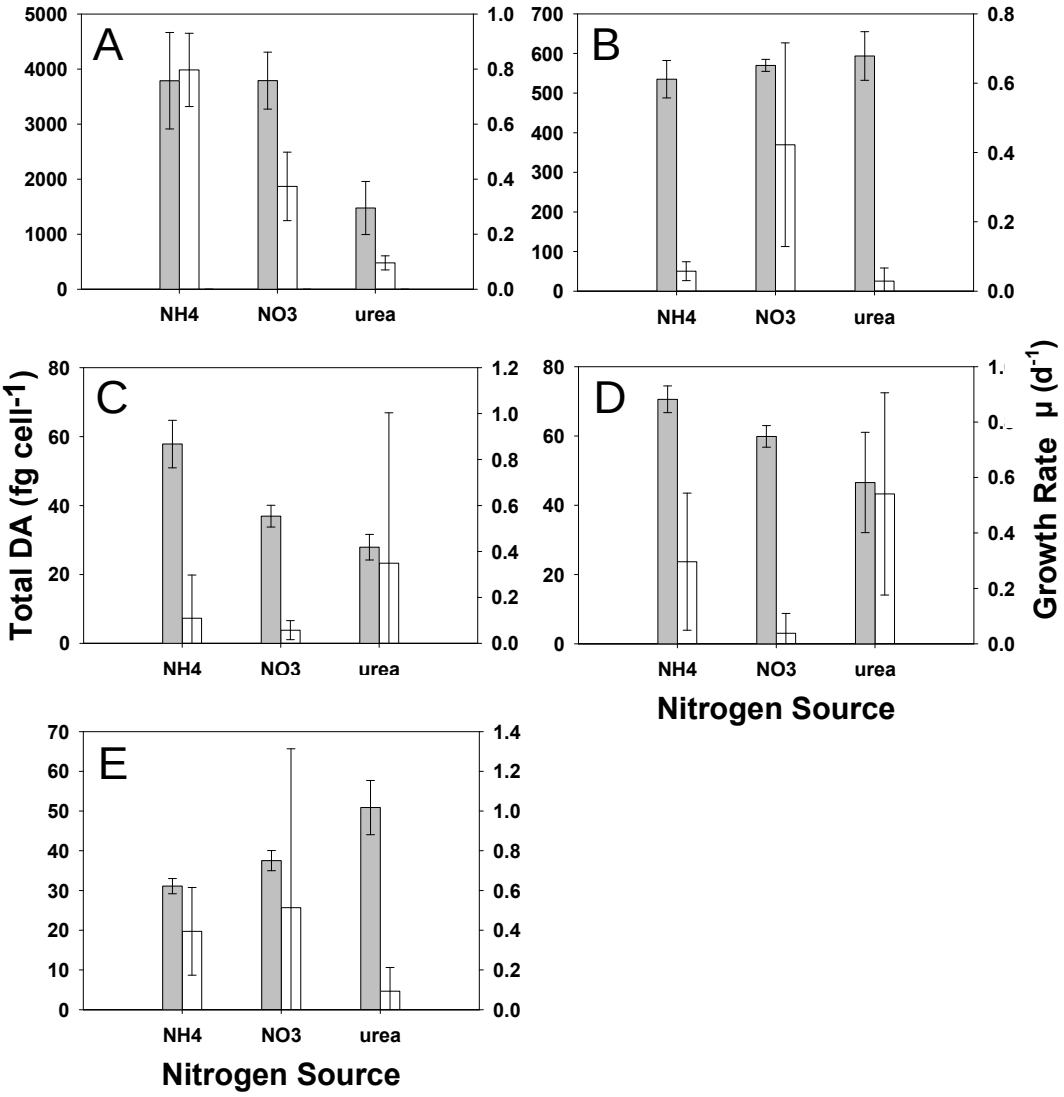
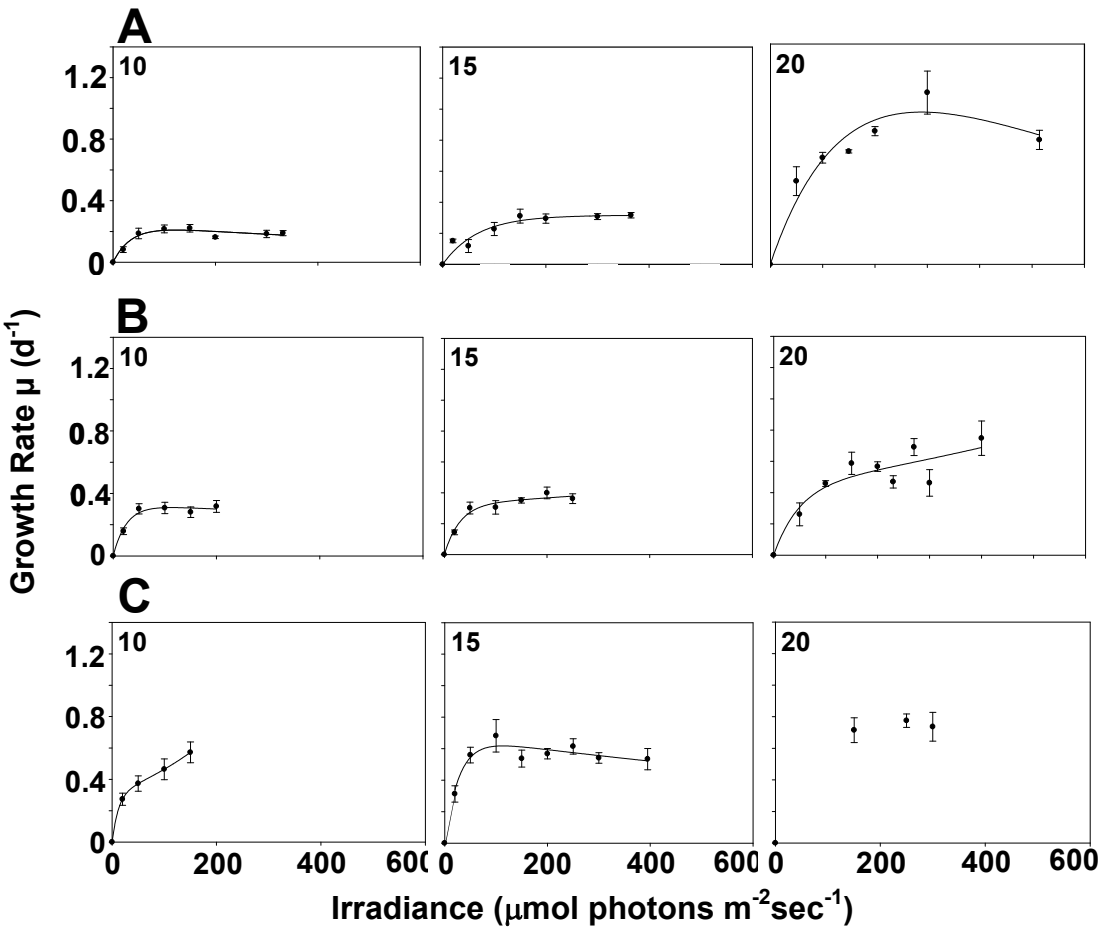

A
B
C
D
E
NH4
NO3
urea
Total DA (fg cell-1)
Growth Rate μ (d-1)
Nitrogen Source

Fig. 10


A
B
C
10
15
20
1.2
0.8
0.4
0
0
200
400
600
Growth Rate μ (d-1)
Irradiance (μmol photons m-2sec-1)