

Iron requirements of the pennate diatom *Pseudo-nitzschia*: Comparison of oceanic (high-nitrate, low-chlorophyll waters) and coastal species

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Abstract

We quantified and compared physiological parameters and iron requirements of several oceanic *Pseudo-nitzschia* spp., newly isolated from the high-nitrate, low-chlorophyll waters of the northeast subarctic Pacific, with coastal *Pseudo-nitzschia* spp. and the oceanic centric diatom *Thalassiosira oceanica* at a range of iron concentrations. In iron-replete conditions, the iron (Fe):carbon (C) ratios in the six *Pseudo-nitzschia* isolates ranged from 157 $\mu\text{mol Fe mol C}^{-1}$ to 248 $\mu\text{mol Fe mol C}^{-1}$, with no apparent differences between oceanic and coastal isolates. In low iron conditions, all *Pseudo-nitzschia* spp. exhibited marked reductions in photosynthetic efficiency, whereas the extent of the reductions in specific growth rates varied among species. When iron-limited, the Fe:C ratios decreased significantly in all oceanic *Pseudo-nitzschia* species, with the lowest ratios ranging from 2.8 $\mu\text{mol Fe mol C}^{-1}$ to 3.7 $\mu\text{mol Fe mol C}^{-1}$. Combined with faster growth rates, lower Fe:C ratios in oceanic isolates of *Pseudo-nitzschia* resulted in significantly higher iron-use efficiencies relative to their coastal congeners and *T. oceanica*. The wide range between iron-replete ($\text{Fe-Q}_{\text{high}}$) and iron-limited (Fe-Q_{low}) quotas indicates that oceanic *Pseudo-nitzschia* spp. have an extensive plasticity in iron contents relative to other diatoms grown at similar iron concentrations reported in the literature; the $\text{Fe-Q}_{\text{high}}:\text{Fe-Q}_{\text{low}}$ ratios for oceanic species were 46 to 67, whereas for coastal *Pseudo-nitzschia* species they were 16 and 43. We suggest that the ability of oceanic *Pseudo-nitzschia* species to exhibit an extensive growth response to iron enrichment events may, in part, be a result of their extraordinary capacity to accumulate and potentially store large amounts of intracellular iron when iron concentrations are high, yet substantially reduce their iron requirements to sustain fast growth rates well after external iron concentrations are depleted.

Low iron concentrations in extensive regions of the world's oceans regulate phytoplankton growth and macronutrient utilization and have a pronounced impact on ocean primary productivity and biogeochemistry. Iron

fertilization studies in the equatorial, north Pacific, and southern oceans provide compelling evidence that iron enrichment of high-nitrate, low-chlorophyll (HNLC) waters results in a dramatic increase in phytoplankton growth and depletion of macronutrient inventories in surface waters, thus confirming the iron limitation hypothesis (Martin et al. 1994; Coale et al. 1996; Boyd et al. 2000; Tsuda et al. 2003; Boyd et al. 2004; Coale et al. 2004). These iron-induced, open ocean blooms have been typically dominated by large, fast-growing diatom species even though their ambient abundances are low relative to smaller, non-diatom phytoplankton populations.

In most of the iron-enrichment experiments, large pennate diatoms, such as *Pseudo-nitzschia*, responded most favorably and numerically dominated post-iron-enriched diatom assemblages (Gervais et al. 2002; Coale et al. 2004; Marchetti et al. in press). *Pseudo-nitzschia* is a ubiquitous diatom genus comprising roughly 29 species (Lundholm et al. 2002) and has been documented in tropical to polar waters (Hasle 2002). Members often persist in open ocean waters as well as turbulent coastal waters, demonstrating their ability to tolerate a wide range of marine environments. The growth characteristics and nutrient require-

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ments of coastal variants of the genus *Pseudo-nitzschia* have been well studied because of their production of the neurotoxin domoic acid (DA). Of these studies, only a few have investigated the physiology of *Pseudo-nitzschia* in relation to iron nutrition (Bates et al. 2001; Maldonado et al. 2002; Wells et al. 2005). Moreover, the growth characteristics and iron requirements of oceanic *Pseudo-nitzschia* have, thus far, never been thoroughly examined.

The ability of oceanic phytoplankton to maintain higher growth rates at low iron concentrations when compared to coastal varieties has been well established. Brand (1991) reported lower iron requirements in oceanic phytoplankton when comparing 10 oceanic to 5 coastal species from a variety of phytoplankton groups. Similar findings were observed by Sunda et al. (1991), who demonstrated oceanic phytoplankton have a six- to eight-fold lower iron requirement than coastal phytoplankton, and by Maldonado and Price (1996), who compared oceanic and coastal species of the centric diatom *Thalassiosira*. When iron availability is low, phytoplankton commonly adopt a number of strategies to minimize their iron demands. These include reducing cell size (Brand et al. 1983; Sunda and Huntsman 1995; Maldonado and Price 1996), replacing iron-containing enzymes and proteins with iron-free equivalents (La Roche et al. 1993), and expressing an inducible high-affinity iron transport system (Harrison and Morel 1986; Maldonado and Price 2001). Recent evidence also indicates a fundamental distinction in photosynthetic architecture between oceanic and coastal diatoms. Oceanic diatoms contain lower photosystem I and cytochrome *b₆f* complex concentrations, which are both iron-rich photosynthetic components (Strzepek and Harrison 2004). Yet, despite these adaptations, iron concentrations in some marine environments are still below the levels required to achieve maximal growth for many phytoplankters. Consequently, iron-limited phytoplankton cells have reduced photosynthetic efficiency and rates of cell division.

To date, the majority of studies on iron-related physiological processes in oceanic diatoms have been performed using centric diatoms isolated from non-iron-limited regions. Only within the last decade have investigators made attempts to specifically isolate and examine truly oceanic diatoms from HNLC waters (Muggli et al. 1996; Timmermans et al. 2001). This is primarily because most oceanic diatoms are difficult to maintain in culture. Thus, despite the prevalence of large pennate diatoms in iron-limited regions, little is known of their physiology and elemental compositions. In addition, it is unlikely that the measured responses to low iron concentrations in previously examined centric diatoms can be extrapolated to pennate diatoms, which possess distinct morphologies and are likely to have different nutrient uptake kinetics (Pahlow et al. 1997).

In this study, we examined the changes in growth rates and intracellular iron quotas in six isolates of the pennate diatom *Pseudo-nitzschia* at a range of iron concentrations. Oceanic and coastal *Pseudo-nitzschia* species were compared to assess their abilities to acclimate to the low-iron conditions. In addition, growth rates and iron requirements

of the oceanic *Pseudo-nitzschia* spp. were compared to the commonly examined oceanic centric diatom, *Thalassiosira oceanica*.

Materials and methods

Algal species—Six isolates (at least five different species) of the pennate diatom *Pseudo-nitzschia* were examined for this study (Table 1). Four oceanic isolates, *Pseudo-nitzschia* cf. *turgidula* (UBC 103), *Pseudo-nitzschia dolorosa* (UBC 203), *Pseudo-nitzschia heimii* type 1 (UBC 403), and *Pseudo-nitzschia* cf. *heimii* type 2 (UBC 303) were obtained from iron-enriched (2–4 nM) seawater samples from Ocean Station Papa ([OSP] 145°W, 50°N) in the northeast Pacific Ocean. Iron concentrations at OSP are typically subnanomolar, whereas concentrations of other macronutrients (nitrate, phosphate, and silicic acid) are consistently high, characterizing this region as HNLC (Harrison 2002). Morphometric characteristics of the oceanic *Pseudo-nitzschia* isolates are provided in Marchetti (2005). Two coastal *Pseudo-nitzschia* species were also examined; *Pseudo-nitzschia* cf. *calliantha* (NWFSC186) was isolated from the Juan de Fuca Eddy in the northeast Pacific and *Pseudo-nitzschia multiseriis* (Orø13) was isolated from Isefjord, Denmark. The specific growth rates and iron requirements of the oceanic centric diatom *Thalassiosira oceanica* (CCMP 1003), isolated from the Sargasso Sea, were also measured. *T. oceanica* (1003) was obtained from the Canadian Centre for the Culture of Microorganisms at the University of British Columbia, Canada.

Medium and culture conditions—All phytoplankton cultures were grown in natural seawater collected from OSP using trace metal clean (TMC) techniques. Seawater was collected from 10–15 m using Teflon tubing connected to a Teflon Bellows pump and brought directly into a TMC positive-pressure laminar flowhood. Seawater was passed through a 0.2- μ m cartridge filter and stored in acid-washed (1 mol L⁻¹ HCl), Q-H₂O (Millipore, >18 M Ω cm⁻¹)-rinsed, 20-liter polycarbonate carboys. The carboys were double bagged and stored in the dark at 4°C. The ambient total iron (Fe_T) and macronutrient concentrations of the OSP seawater were 0.06 nmol L⁻¹ Fe_T, 15 μ mol L⁻¹ NO₃, 22 μ mol L⁻¹ Si(OH)₄, and 1.3 μ mol L⁻¹ PO₄. In the laboratory, the OSP seawater was stored in the dark at room temperature inside a TMC room. All dispensing of seawater was performed under a laminar flowhood. Low-iron natural seawater was used for the culture medium rather than artificial seawater medium (such as AQUIL) because of the lower background concentrations of iron achievable in the OSP-based medium.

For medium preparation, 1- or 2-liter aliquots of OSP seawater were placed into acid-washed, Q-H₂O-rinsed polycarbonate bottles. For low-iron treatments, the polycarbonate bottles were also soaked in TMC 1 mol L⁻¹ HCl (Seastar Baseline) for at least 48 h before rinsing with Q-H₂O. Dispensed OSP seawater was sterilized by microwaving. The medium was then cooled and supplemented with filter-sterilized (0.2 μ m Acrodisc) ethylenediaminetetraacetic acid (EDTA)-trace metals (minus iron), vitamins (B₁₂,

Table 1. *Pseudo-nitzschia* species used in this study along with strain designations, sampling locations and dates, and isolators.

<i>Pseudo-nitzschia</i> spp.	Strain	Isolation location and date	Isolator
Oceanic			
<i>P. cf. turgidula</i>	UBC103	Ocean Station Papa, NE Pacific (50.0°N, 145.0°W) Sep 2003	A. Marchetti
<i>P. dolorosa</i>	UBC203	Ocean Station Papa, NE Pacific (50.0°N, 145.0°W) Sep 2003	A. Marchetti
<i>P. heimii</i> type 1	UBC403	Ocean Station Papa, NE Pacific (50.0°N, 145.0°W) Sep 2003	A. Marchetti
<i>P. cf. heimii</i> type 2	UBC303	Ocean Station Papa, NE Pacific (50.0°N, 145.0°W) Sep 2003	A. Marchetti
Coastal			
<i>P. multiseriis</i>	Orø13	Isefjord, Denmark Aug 2001	L. Holtegaard
<i>P. cf. calliantha</i>	NWFSC186	Juan de Fuca Eddy, Washington, USA (48.5°N, 125.5°W) Aug 2004	B. Bill

thiamine, and biotin), and pre-chelexed macronutrients [NO₃, Si(OH)₄, and PO₄] in accordance with AQUIL medium concentrations (Price et al. 1988/89). In addition to ambient OSP macronutrient concentrations, nutrients were supplied to OSP medium in final concentrations of 300 μmol L⁻¹ NO₃, 100 μmol L⁻¹ Si(OH)₄, and 10 μmol L⁻¹ PO₄. Trace metal concentrations were buffered using 100 μmol L⁻¹ of EDTA. The free ferric ion (Fe³⁺) activities were estimated with the MINEQL model (version 2.0, Westall et al. 1976), assuming the concentrations of the major EDTA-binding cations (e.g., Ca²⁺ and Mg²⁺) in the OSP-based medium were similar to those used in AQUIL (Price et al. 1988/89). This ignores the presence of natural organic Fe(III)-binding ligands or reduced Fe(II) species, such that the iron is allocated only between the dominant Fe(III)-EDTA pool, the secondary pool of inorganic-bound species, and the remaining very small pool of truly free Fe³⁺ ion. Premixed Fe-EDTA (1:1) was added separately at a total concentration of 1.37 μmol L⁻¹, 6.2 nmol L⁻¹, or 3.1 nmol L⁻¹ to achieve Fe³⁺ concentrations of 10⁻¹⁹ mol L⁻¹ (pFe 19), 10^{-21.35} mol L⁻¹ (pFe 21.4), or 10^{-21.65} mol L⁻¹ (pFe 21.7), respectively. The OSP medium was allowed to equilibrate chemically overnight before use and was stored in a TMC room. Cultures were grown in pFe 19 medium to obtain iron-replete growth, whereas pFe 21.4 and pFe 21.7 media were used to obtain iron-stressed or iron-limited growth.

Duplicate phytoplankton cultures were grown and maintained in acid-washed, 28-mL polycarbonate centrifuge tubes at 12°C (for *Pseudo-nitzschia* spp.) or 18°C (for *T. oceanica*) under a continuous, saturating photon flux density of 166 μmol photons m⁻² s⁻¹, using the semi-continuous batch culture technique described by Brand et al. (1981). Although sterile techniques were used for all culture work to minimize bacterial contamination, cultures were not considered axenic.

Specific growth rates—Phytoplankton growth rates were estimated by directly measuring *in vivo* chlorophyll *a* (Chl *a*) fluorescence using a Turner Designs model 10-AU fluorometer (in vivo chlorophyll optical kit). Specific growth rates (μ) were calculated from the linear regression

of the natural log of *in vivo* fluorescence versus time during the exponential growth phase of acclimated cells after confirming a proportional relationship between fluorescence and cell concentration. Phytoplankton growth rates were considered in steady-state when rates of successive transfers did not vary by more than 10% (typically 4–5 transfers). The *r*² values of *in vivo* fluorescence versus cell concentration standard curves for several *Pseudo-nitzschia* species at each iron treatment were >0.96 (data not shown).

Photosystem II maximum photochemical quantum yield—As a proxy for iron-stress, fluorescence induction measurements were performed on cultures in mid-exponential phase using a pulse amplitude modulated (PAM) 101/103 fluorometer equipped with an ED-101PM emitter-detector-cuvette (Walz). Before each measurement, a subsample (5 mL) of the culture was dark acclimated at 12°C for 20 min (Schreiber et al. 1995). The constant fluorescence of dark-adapted cells (*F*₀) was measured using a modulated light (US-L655, Walz) at a low intensity (5 μmol photons m⁻² s⁻¹) to avoid reduction of the photosystem II (PSII) primary electron acceptors. The maximal fluorescence yield (*F*_m) was induced by a short (700 ms) saturating pulse of light (3,000 μmol photons m⁻² s⁻¹), which triggered the reduction of all PSII plastoquinone pools. The PSII maximum photochemical quantum yield (Φ_M) was then calculated as described in Schreiber et al. 1986 after the subtraction of a 0.2-μm filtered seawater blank.

Iron-to-carbon ratios—Intracellular iron:carbon (Fe:C) ratios were determined using an ⁵⁵Fe and ¹⁴C dual label, radiotracer technique (Tortell et al. 1996). For these experiments, OSP medium was prepared in acid-washed, 1-liter polycarbonate bottles as described previously. Ambient iron concentrations in OSP media were assumed to be negligible (Fe_T < 0.1 nmol L⁻¹). For iron-replete medium (pFe 19), 13.7 nmol L⁻¹ of ⁵⁵FeCl₃ was added along with 1,356 nmol L⁻¹ non-radio-labeled FeCl₃ so that ⁵⁵Fe was 1% of Fe_T. For the low-iron medium, 6.2 nmol L⁻¹ (pFe 21.4) and 3.1 nmol L⁻¹ (pFe 21.7) of ⁵⁵FeCl₃ was added so that 100% of Fe_T was radio-labeled. All iron

additions were pre-mixed with EDTA (1 : 1) in TMC Teflon vials and allowed to equilibrate in the medium overnight. In addition, 20 μCi of $\text{H}^{14}\text{CO}_3^-$ was added to each iron treatment in the 1-liter polycarbonate bottles to enable the simultaneous measurement of intracellular iron and carbon.

To initiate experiments, 50 μL (ca. 1000 cells) of late-exponential phase culture that was pre-acclimated to the specific iron concentration was inoculated into an acid-washed, 28-mL polycarbonate centrifuge tube containing the pFe equivalent radio-labeled medium. This inoculum cell density permitted at least eight cell divisions to occur before harvesting to ensure >99% of the cells were radio-labeled. Growth rates were measured using *in vivo* fluorescence as described previously. When cultures reached the mid- to late-exponential phase, the cells were harvested onto polycarbonate filters (2- μm pore size) and soaked for 5 min with titanium-EDTA-citrate reducing solution followed by a filtered seawater rinse to remove iron adsorbed onto the cell surface (Hudson and Morel 1989). Incorporated ^{55}Fe and ^{14}C were then measured with a liquid scintillation counter (Beckman, LS6500). To correct for the interference between ^{55}Fe and ^{14}C radio-nuclides, a dual-labeled quench curve was performed. For each iron treatment, absorption of ^{55}Fe and ^{14}C onto the filter was corrected by filtering 25 mL of cell-free medium. This blank was then subtracted from each measurement.

Results

Growth parameters—The physiological status of each isolate in relation to iron nutrition was assessed by measuring growth rates and PSII maximum photochemical quantum yields (Φ_M). In iron-replete conditions (pFe 19), the maximum specific growth rates (μ_{max}) among the oceanic *Pseudo-nitzschia* isolates ranged from 1.10 d^{-1} (*P. cf. turgidula*) to 1.35 d^{-1} (*P. cf. heimii* type 2) (Fig. 1A). Between the coastal *Pseudo-nitzschia* isolates, μ_{max} were similar (0.9–1.0 d^{-1}) and ca. 20% slower than oceanic isolates (Fig. 1B). Although for some phytoplankton species the presence of high EDTA concentrations in the medium may be deleterious to growth (Muggli and Harrison 1996), the μ_{max} of the *Pseudo-nitzschia* species examined in this study did not markedly differ from the μ_{max} of oceanic and coastal *Pseudo-nitzschia* spp. reported by Lundholm et al. (2004), which were grown in the presence of 11.7 $\mu\text{mol L}^{-1}$ EDTA. Therefore, the ca. nine-fold higher EDTA concentration used in this study did not appear to negatively affect *Pseudo-nitzschia* growth.

Iron-limited conditions were examined using two low-iron concentrations (pFe 21.4 or pFe 21.7). At pFe 21.4, four out of the six *Pseudo-nitzschia* isolates exhibited significant reductions in specific growth rates compared to iron-replete conditions (Student's *t*-test, $p < 0.05$, for *P. cf. heimii* type 2, *P. cf. turgidula*, *P. cf. calliantha*, and *P. multiseriis*), whereas at pFe 21.7, growth rates of all *Pseudo-nitzschia* isolates except for *P. dolorosa* were significantly different (Student's *t*-test, $p < 0.05$). The greatest reduction in growth rate among oceanic *Pseudo-nitzschia* isolates occurred in *P. cf. turgidula*, where μ

declined 1.9-fold at pFe 21.7 relative to iron-replete conditions. Both coastal *Pseudo-nitzschia* isolates also exhibited extensive reductions in μ at low-iron conditions, with *P. multiseriis* and *P. cf. calliantha* exhibiting a decrease in μ of 1.9-fold (pFe 21.4) and 1.6-fold (pFe 21.7), respectively. At pFe 21.7, cultures of *P. multiseriis* did not grow because of severe iron limitation. Similarly, μ of *T. oceanica* were significantly reduced (1.6-fold) in low iron conditions (pFe 21.7) when compared to iron-replete conditions (Student's *t*-test, $p < 0.05$) (Fig. 1C).

The decrease in PSII maximum photochemical yields in all examined isolates under low-iron conditions clearly indicates reduced photosynthetic efficiencies as a result of iron-limited growth. In general, at pFe 21.4 and 21.7, Φ_M values declined more in oceanic isolates (ranging from 19% in *P. dolorosa* to 83% in *P. cf. heimii* type 2) when compared to coastal *Pseudo-nitzschia* isolates and *T. oceanica* (Fig. 1D–F).

Intracellular Fe : C ratios and iron-use efficiencies—In iron-replete conditions, the Fe : C ratios of all *Pseudo-nitzschia* species ranged from 157 $\mu\text{mol Fe mol C}^{-1}$ to 248 $\mu\text{mol Fe mol C}^{-1}$ with no significant differences observed between the oceanic and coastal *Pseudo-nitzschia* isolates examined (ANOVA, $p > 0.05$, $F_{5,15} = 2.90$) (Table 2). The variability in Fe : C ratios in iron-replete conditions was generally high not only among isolates, but also among replicates within each diatom. At low-iron conditions, cellular Fe : C ratios of all isolates decreased markedly compared to those of iron-replete cells. At pFe 21.4, the Fe : C ratios of the oceanic isolates ranged from 4.1 $\mu\text{mol Fe mol C}^{-1}$ in *P. cf. turgidula* to 7.2 $\mu\text{mol Fe mol C}^{-1}$ in *P. dolorosa*. At pFe 21.4, the Fe : C ratios in coastal *Pseudo-nitzschia* isolates were significantly higher compared to the oceanic isolates (ANOVA, $p < 0.05$, $F_{5,17} = 2.81$, Tukey test). At pFe 21.7, the Fe : C ratios of the oceanic isolates ranged from 2.8 $\mu\text{mol Fe mol C}^{-1}$ in *P. cf. turgidula* and *P. heimii* type 1 to 3.7 $\mu\text{mol Fe mol C}^{-1}$ in *P. dolorosa*. In the coastal isolates, only the Fe : C ratio of *P. cf. calliantha* could be measured because of the inability of *P. multiseriis* to achieve acclimated growth at pFe 21.7. In this low iron condition, the Fe : C ratio of *P. cf. calliantha* was 5.2 $\mu\text{mol Fe mol C}^{-1}$, which was significantly higher than the oceanic *Pseudo-nitzschia* isolates (ANOVA, $p < 0.05$, $F_{5,14} = 2.96$, Tukey test). Similarly, at pFe 21.7, the Fe : C ratio of *T. oceanica* (5.4 $\mu\text{mol Fe mol C}^{-1}$) was also significantly higher than the oceanic *Pseudo-nitzschia* isolates (ANOVA, $p < 0.05$, $F_{5,14} = 2.96$, Tukey test).

Iron-use efficiency (IUE) is defined as the rate of assimilated carbon per unit of cellular iron. The IUEs of all *Pseudo-nitzschia* isolates increased when grown in low iron conditions (Table 2). The IUEs of oceanic isolates were markedly higher than those of the coastal isolates in both low iron conditions. On average, the maximum IUEs for oceanic and coastal *Pseudo-nitzschia* spp. in low-iron conditions (pFe 21.4 for *P. multiseriis* and pFe 21.7 for all other *Pseudo-nitzschia* spp.) were $3.2 \times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$ and $1.0 \times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$ respectively, representing a three-fold difference. In addition, at pFe 21.7, the maximum IUEs of the oceanic *Pseudo-nitzschia*

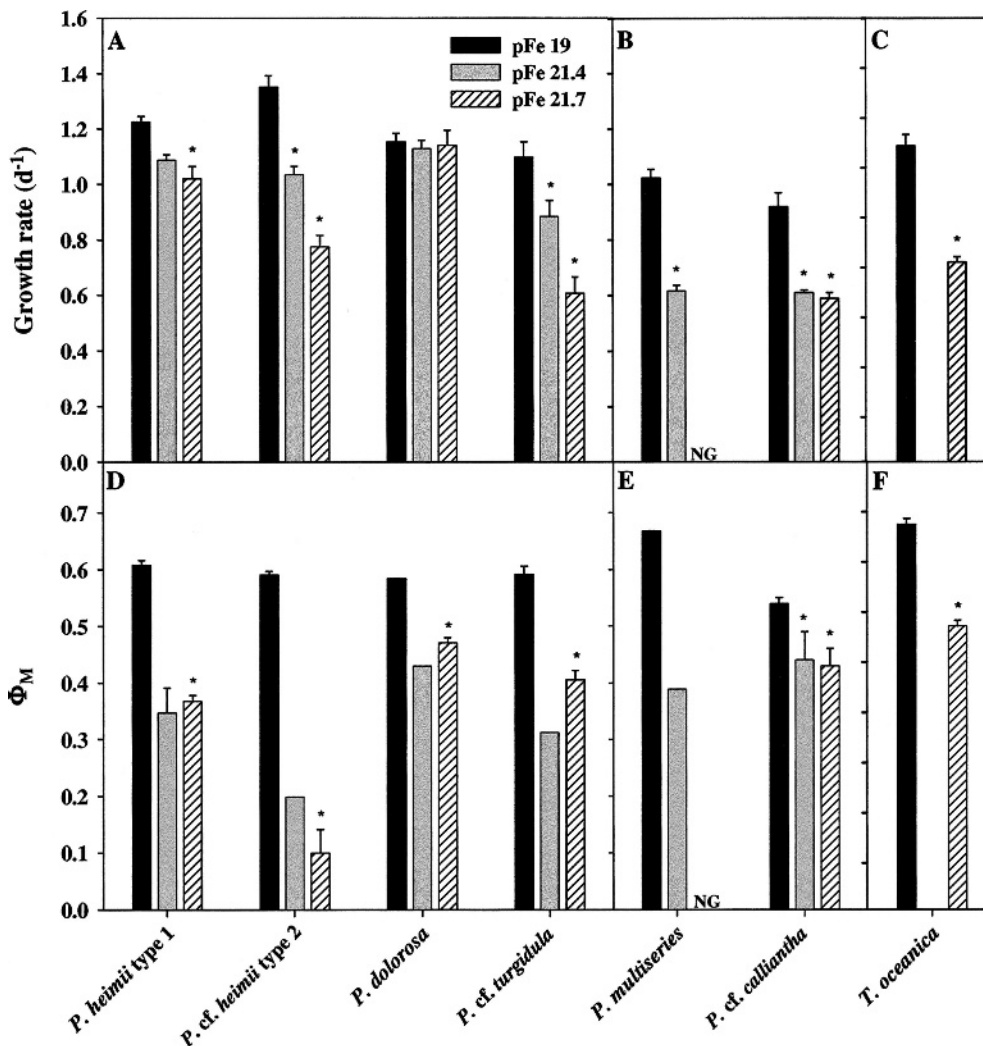


Fig. 1. Specific growth rates (A, B, and C) and PSII maximum photochemical quantum yields (Φ_M) (D, E, and F) of oceanic (A and D), coastal (B and E) *Pseudo-nitzschia* species and *T. oceanica* (C and F) grown in iron-replete [total iron (Fe_T) = 1,370 nmol L⁻¹ (pFe 19)] and low iron conditions [Fe_T = 6.2 nmol L⁻¹ (pFe 21.4) and 3.1 nmol L⁻¹ (pFe 21.7)]. For growth rates (A, B, and C), error bars represent ± 1 standard error associated with the mean ($n \geq 3$, see Table 2). NG = no growth. For Φ_M (D, E, and F), where provided, error bars represent ± 1 standard error associated with the mean ($n \geq 3$, for pFe 21.4 treatments $n = 1$ except for *P. heimii* type 1, where $n = 2$). Low iron treatments that are significantly different (Student's *t*-test, $p < 0.05$) from the iron-replete treatment are indicated with an asterisk.

spp. were significantly higher than the IUE measured in *T. oceanica* (1003) (ANOVA, $p < 0.05$, $F_{4,13} = 3.18$, Tukey test).

Discussion

Oceanic Pseudo-nitzschia spp. growth rates and iron requirements—To our knowledge, our study is the first assessment of physiological characteristics and iron requirements of the ecologically important genus *Pseudo-nitzschia*, isolated from HNLC waters. Most laboratory studies assessing the iron requirements and iron-dependent physiological processes in oceanic diatoms have been performed using centric diatoms such as *Thalassiosira*.

This is despite *Thalassiosira* rarely being a prominent member of post-iron enrichment oceanic diatom assemblages, thus limiting the applicability of using its elemental stoichiometry in ecosystem models such as those assessing global iron budgets (Fung et al. 1997) or biogeochemical cycling of iron (Parekh et al. 2004). To evaluate the full potential of oceanic diatoms to subsist in low-iron environments, it is critical to examine a wide variety of both centric and pennate diatom species, particularly those isolated from HNLC waters where iron limitation is more pronounced.

Reductions in photosynthetic efficiency, as interpreted from decreases in PSII maximum photochemical quantum yields, precede the decline in growth rates when cells are

Table 2. Mean specific growth rates, Fe:C ratios, and iron-use efficiencies of examined *Pseudo-nitzschia* spp. and *T. oceanica*. Cultures were grown in iron-replete (pFe 19) and low-iron (pFe 21.4 or 21.7) conditions. Errors represent ± 1 standard error associated with the mean.

Diatom	[Fe] _T (nmol L ⁻¹)	pFe*	Growth rate (d ⁻¹)	<i>n</i> †	Fe:C ratio ($\mu\text{mol Fe mol C}^{-1}$)	<i>n</i> ‡	Iron-use efficiency§ ($\times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$)
<i>Pseudo-nitzschia</i> species							
Oceanic							
<i>P. heimii</i> type 1	1,370	19	1.23 \pm 0.03	21	161.5 \pm 8.3	4	0.08 \pm 0.003
	6.2	21.4	1.09 \pm 0.03	27	5.8 \pm 0.3	4	2.22 \pm 0.21
	3.1	21.7	1.02 \pm 0.04	22	2.8 \pm 0.2	5	4.02 \pm 0.25
<i>P. cf. heimii</i> type 2	1,370	19	1.35 \pm 0.04	22	156.5 \pm 4.5	3	0.08 \pm 0.005
	6.2	21.4	1.04 \pm 0.03	26	5.7 \pm 0.4	5	1.96 \pm 0.11
	3.1	21.7	0.80 \pm 0.06	19	3.4 \pm 0.1	4	3.24 \pm 0.22
<i>P. dolorosa</i>	1,370	19	1.15 \pm 0.04	17	248.3 \pm 11.9	4	0.05 \pm 0.005
	6.2	21.4	1.13 \pm 0.04	17	7.2 \pm 1.8	3	1.24 \pm 0.18
	3.1	21.7	1.12 \pm 0.05	23	3.7 \pm 0.2	5	3.16 \pm 0.18
<i>P. cf. turgidula</i>	1,370	19	1.10 \pm 0.05	20	186.1 \pm 22.0	3	0.04 \pm 0.003
	6.2	21.4	0.84 \pm 0.05	15	4.1 \pm 0.3	3	2.13 \pm 0.17
	3.1	21.7	0.58 \pm 0.08	10	2.8	1	2.33
Coastal							
<i>P. multiseriis</i>	1,370	19	1.01 \pm 0.03	25	176.4 \pm 19.8	5	0.06 \pm 0.006
	6.2	21.4	0.53 \pm 0.03	18	11 \pm 0.9	4	0.48 \pm 0.07
<i>P. cf. calliantha</i>	1,370	19	0.92 \pm 0.05	12	221.5 \pm 26.5	3	0.05 \pm 0.006
	6.2	21.4	0.61 \pm 0.01	3	10.5 \pm 0.6	3	1.03 \pm 0.05
	3.1	21.7	0.59 \pm 0.02	4	5.2 \pm 0.2	3	1.51 \pm 0.18
<i>T. oceanica</i>	1,370	19	1.14 \pm 0.04	8	114.6 \pm 3.88	3	0.09 \pm 0.005
	3.1	21.7	0.72 \pm 0.02	15	5.4 \pm 0.1	3	1.24 \pm 0.04

* pFe = $-\log[\text{Fe}^{3+}]$.

† Number of growth rates from successive transfers used to generate mean specific growth rate.

‡ Number of replicates used to estimate the Fe:C ratios.

§ Mean iron-use efficiencies listed are the average of independent iron-use efficiencies calculated using the *n* Fe:C ratios and corresponding growth rates.

grown at moderately low iron concentrations. Iron deficiency directly affects the PSII reaction centers by reducing the chlorophyll content and reducing the fitness of electron transport and related downstream processes (Greene et al. 1991). In coastal *Pseudo-nitzschia* isolates and *T. oceanica*, the reductions in Φ_M were, for the most part, coupled to reductions in μ . In contrast, in the oceanic *Pseudo-nitzschia* isolates, there were substantial reductions in Φ_M despite having little or no change in μ . The appreciable decoupling of μ from Φ_M suggests that these oceanic diatoms have either very low energy requirements or an effective mechanism to compensate for this impairment in photosynthetic efficiency to generate reducing power and maintain rapid growth.

The oceanic *Pseudo-nitzschia* species had significantly higher IUEs compared to the coastal species. Because cellular carbon content among *Pseudo-nitzschia* isolates did not change markedly with their iron nutritional status (Marchetti 2005), lower Fe:C ratios in low-iron conditions were primarily a consequence of their reduced intracellular iron content. Most of the IUEs measured for the oceanic *Pseudo-nitzschia* isolates fell within the range reported for a number of other oceanic phytoplankton species ($3\text{--}6 \times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$, Maldonado and Price 1996). In contrast, *T. oceanica* (1003) had a lower iron-use efficiency compared to the oceanic *Pseudo-nitzschia* species isolated from iron-limited waters of the northeast subarctic Pacific, suggesting that oceanic *Pseudo-nitzschia* are better adapted to growing at lower iron concentrations. It should be noted

that the IUE that we measured for *T. oceanica* (1003) was appreciatively lower than the value reported in Maldonado and Price (1996) ($1.2 \pm 0.04 \times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$ versus $2.5 \pm 0.01 \times 10^5 \text{ mol C mol Fe}^{-1} \text{ d}^{-1}$). The difference in IUE for *T. oceanica* (1003) was a consequence of a slower μ under our low-iron conditions rather than variability in the Fe:C ratio. In fact, the minimum Fe:C ratio reported for *T. oceanica* (1003) by Maldonado and Price (1996) is in good agreement to the minimum Fe:C ratio measured in our study (4.9 ± 0.1 versus $5.4 \pm 0.1 \mu\text{mol Fe mol C}^{-1}$), despite being grown at different low iron concentrations. Thus, the slower μ measured in *T. oceanica* (1003) in our study translated to a lower IUE. We also observed higher Fe:C ratios in iron-replete *T. oceanica* (1003) when compared to these previous studies. Reasons for this discrepancy are under investigation at the present time.

Our results, those of other laboratory studies, and measurements performed using natural phytoplankton assemblages all provide compelling evidence for highly dynamic intracellular Fe:C ratios in marine diatoms (Sunda and Huntsman 1995; Maldonado and Price 1996; Twining et al. 2004). Under steady-state conditions the intracellular quota (*Q*) of a particular limiting nutrient follows a well established hyperbolic relationship across a range of external nutrient concentrations (*S*) (Droop 1973; Morel 1987). As defined by Droop (1968), when μ approaches zero, the subsistence quota (k_Q), which is the minimum nutrient quota required to maintain cell growth,

may be determined. In our study, although growth rates were reduced because of iron limitation, the extent of these reductions was not severe. Therefore, additional decreases in iron concentrations below pFe 21.7 may yield further declines in μ and subsequently lower Fe:C ratios until Fe- k_Q is approached. The subsistence quota may also vary with experimental growth conditions (e.g., light intensity and temperature) and chemical composition of the medium (e.g., nitrogen source) (Strzepek and Price 2000).

When external nutrient concentrations are high, the intracellular quota may continue to increase above the quota where μ_{\max} is achieved until the maximum quota (Q_m) is obtained, which is defined by the asymptote of the previously described Q versus S relationship. Between the two low-iron conditions, an ca. 2.2-fold increase in the Fe³⁺ concentration resulted in an averaged 1.8-fold higher Fe:C ratio in the examined *Pseudo-nitzschia* species. In contrast, the maximum increase in the Fe:C ratios between pFe 21.7 and the Fe-replete treatment (pFe 19) was 67-fold (for *P. dolorosa*), despite the ca. 560-fold increase in Fe³⁺ concentration. This observed hyperbolic relationship suggests that Q_m was reached and any additional increase in iron concentrations above pFe 19 would not likely yield significantly higher Fe:C ratios. Thus, we speculate that the high Fe:C ratios reported for each diatom species examined are representative of their maximum iron quotas, which may be interpreted as their iron requirements at μ_{\max} plus their maximum attainable iron storage capacity (see further discussion below).

Fe- Q_{high} :Fe- Q_{low} ratio—In *Thalassiosira* spp., a trade-off was observed between oceanic variants, which had lower iron requirements, and coastal variants, which had an increased capacity to accumulate iron when external concentrations were high (Sunda et al. 1991; Sunda and Huntsman 1995). The capacity to take up more iron than required to satisfy biochemical functions for growth at μ_{\max} would be another important iron-acquisition strategy of phytoplankton. Maldonado and Price (1996) estimated this ability by calculating the ratio between the iron quotas of iron-replete and iron-limited diatoms. The resulting high iron quota to low iron quota ratio (Fe- Q_{high} :Fe- Q_{low}) reflects the ability of a phytoplankter to potentially store intracellular iron when dissolved iron concentrations are high, yet lower iron requirements to a minimum when iron concentrations are limiting. Among phytoplankton isolates, the general trend observed was that oceanic species achieved a lower Fe- Q_{low} and coastal species achieved a higher Fe- Q_{high} . The advantages for oceanic species having lower iron quotas have been previously discussed. In contrast, diatoms, particularly bloom-forming species, may benefit from being able to take up and store large quantities of iron during periods of surplus availability because these reserves can then be drawn upon as iron concentrations become exhausted. The relatively high Fe- Q_{high} :Fe- Q_{low} ratios observed in *Thalassiosira* isolates from the HNLC regions of the equatorial Pacific compared to coastal and oceanic *Thalassiosira* isolates were suggested to be a result of these diatoms living in low-iron

environments with sporadic iron inputs (Maldonado and Price 1996; see Table 3).

We calculated the Fe- Q_{high} :Fe- Q_{low} ratio for the oceanic and coastal isolates of *Pseudo-nitzschia* and compared these values to *Thalassiosira* spp. grown at similar ranges of iron-replete and low-iron conditions reported in the literature (Table 3). The Fe- Q_{high} :Fe- Q_{low} ratios for the oceanic *Pseudo-nitzschia* spp. examined were markedly higher than those calculated for oceanic *Thalassiosira* spp. The differences in ratios of the oceanic *Pseudo-nitzschia* isolates compared to oceanic *Thalassiosira* spp. were a consequence of a higher Fe- Q_{high} . Therefore, it appears that the iron quotas of the oceanic *Pseudo-nitzschia* spp. span the entire range of iron quotas exhibited by the oceanic and coastal *Thalassiosira* spp. as they maintained similar or lower minimal iron requirements in low-iron conditions yet had the ability to greatly increase their iron storage capacity when in iron-replete conditions.

The success of diatoms in aquatic environments is often attributed to their ability to rapidly accumulate nutrients beyond their immediate cellular requirements because of the presence of large storage vacuoles (Stolte and Riegman 1995). For example, the accumulation of various nitrogen compounds, which often regulates growth in coastal and oceanic waters, is commonly observed in diatoms (Dortch 1982). However, the capabilities of diatoms to potentially perform “luxury uptake” for trace elements such as iron are not well understood. The maximum iron quotas attainable would be somewhere between the biochemical iron requirements of a phytoplankter needed to support μ_{\max} and their ability to accumulate and store iron while avoiding toxic effects that occur when present at high concentrations. The toxicity of iron is a result of its tendency to form oxygen radicals that may damage cells. To mitigate iron toxicity, many bacteria, plants, and animals have evolved specific iron storage compounds, such as ferritin, which tie up iron and prevent it from damaging other molecules. Such storage compounds enable iron to be readily released and utilized when needed, thus acting as a buffer against iron deficiency. The recent sequencing of the complete genomes of *Thalassiosira pseudonana* and *Phaeodactylum tricorutum* provides additional opportunities for investigating diatom architecture (Armbrust et al. 2004; C. Bowler et al. unpubl. data). Interestingly, the pennate diatom *P. tricorutum* contains a putative ferritin gene, whereas a homolog of genes encoding ferritin has not been identified in *T. pseudonana*, suggesting that a fundamental distinction in iron storage mechanisms may exist between centric and pennate diatoms.

Oceanographic relevance—It has recently been suggested that demoic acid (DA), the compound produced by some coastal members of the genus *Pseudo-nitzschia*, may serve as a metal-complexing organic ligand. Wells et al. (2005) speculated that iron-stressed *Pseudo-nitzschia* spp. increase their production of DA, which is then excreted outside of the cell to complex either iron or copper. This hypothesis builds on two previous studies that demonstrated that DA binds iron and copper (Rue and Bruland 2001) and that the

Table 3. Comparison of Fe:C ratios in oceanic and coastal diatoms of *Pseudo-nitzschia* spp. and *Thalassiosira* spp. grown in iron-replete and low iron conditions.

Diatom	Iron treatment	[Fe] _T * (nmol L ⁻¹)	Fe:C ratio (μmol Fe mol C ⁻¹)	Fe-Q _{high} : Fe-Q _{low} †	References
Oceanic					
<i>P. heimii</i> type 1 (UBC403)	High	1,370	161.5		
	Low	3.1	2.8	57.7	This study
<i>P. cf. heimii</i> type 2 (UBC303)	High	1,370	156.5		
	Low	3.1	3.4	46.0	This study
<i>P. dolorosa</i> (UBC203)	High	1,370	248.3		
	Low	3.1	3.7	67.1	This study
<i>P. cf. turgidula</i> (UBC103)	High	1,370	186.1		
	Low	3.1	2.8	66.2	This study
<i>T. oceanica</i> (13.1)	High	1,010	46.7		
	Low	4.2	3.0	15.6	Sunda and Huntsman, 1995
<i>T. oceanica</i> (1003)	High	1,370	114.6		
	Low	3.1	5.4	21.2	This study
<i>T. oceanica</i> (1003)	High	8,410	29.0		
	Low	12.9	4.9	5.9	Maldonado and Price, 1996
Coastal					
<i>P. multiseriis</i> (Orø13)	High	1,370	176.4		
	Low	6.2	11.0	16.0	This study
<i>P. cf. calliantha</i> (NWSFC186)	High	1,370	221.5		
	Low	6.2‡	10.5	21.1	This study
	Low	3.1	5.2	42.5	
<i>Thalassiosira pseudonana</i> (3H)	High	1,023	149.0		
	Low	9.5	12.9	11.5	Sunda and Huntsman, 1995
<i>Thalassiosira weissflogii</i> (Actin)	High	1,025	101.0		
	Low	11	11.0	7.7	Sunda and Huntsman, 1995
Equatorial Pacific					
<i>Thalassiosira parthenela</i> (Thal 9)	High	8,410	90.0		
	Low	12.9	3.3	27.3	Maldonado and Price, 1996
<i>Thalassiosira subtilis</i> (50 Ait)	High	8,410	56.0		
	Low	12.9	1.7	32.9	Maldonado and Price, 1996

* All growth mediums contained 100 μmol L⁻¹ EDTA.

† Fe-Q_{high}:Fe-Q_{low} represents the ratio of the iron quotas in iron-replete to low-iron conditions.

‡ The Fe:C ratios at Fe_T = 6.2 nmol L⁻¹ (pFe 21.4) are shown to compare iron quotas with *Thalassiosira* spp.

presence of DA in the growth medium enhances rates of iron uptake in both iron-replete and iron-limited cultures of coastal *Pseudo-nitzschia* species (Maldonado et al. 2002). From these studies, it would follow that the ability to produce DA would provide a particular advantage to *Pseudo-nitzschia* spp. residing in low iron environments. Thus, oceanic *Pseudo-nitzschia* species from HNLC waters should have a greater capacity to produce DA to facilitate iron uptake. Although the ability of *Pseudo-nitzschia* species isolated from HNLC regions to produce DA has yet to be confirmed, we propose that an additional mechanism that enables them to persist in low iron environments is through their exceptional ability to modify their cellular iron contents. In HNLC regions where iron concentrations are low, acclimation to growing with a reduced iron requirement would enable oceanic *Pseudo-nitzschia* species to maintain a viable seed population. After sporadic inputs of iron by either aeolian deposition or intermittent upwelling and mixing, the increased iron storage capacity in *Pseudo-nitzschia* cells would permit them to bloom and maintain elevated growth for a longer period of time relative to other diatoms. Such an ability

may explain observations made during the Subarctic Ecosystem Response to Iron Enrichment Study (SERIES) in the northeast subarctic Pacific where, during the post-iron enrichment phytoplankton bloom, *Pseudo-nitzschia* spp. maintained rapid growth rates in the latter stages of the bloom, well after other centric diatom genera had reached senescent phase because of a combination of iron and silicic acid stress (Marchetti et al. in press). In conclusion, we suggest that the broad plasticity in iron quotas among *Pseudo-nitzschia* species grown in iron-replete and low-iron conditions may explain their dominance across a wide range of iron concentrations in the natural environment, particularly during iron-induced algal blooms in HNLC regions.

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