Effects of iron and nitrogen source on the sinking rate, physiology and metal composition of an oceanic diatom from the subarctic Pacific

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ABSTRACT: The effect of iron (Fe) on the sinking rate of an oceanic diatom *Actinocyclus* sp. and an oceanic coccolithophore *Emiliania huxleyi*, both isolated from the subarctic Pacific, was examined in natural oceanic seawater. The Fe status of the diatom had a dramatic effect on its sinking rate, causing a 5 times increase from 0.17 to 0.93 m d^{-1} from Fe-replete to Fe-stressed conditions. In contrast, Fe had no effect on the sinking rate of the oceanic coccolithophore, which maintained its sinking rate at 0.12 m d^{-1} The cell volume of the diatom decreased slightly under Fe-stressed conditions, but the cell volume of the coccolithophore decreased substantially (46%) under Fe-stressed conditions. The effect of nitrogen source (nitrate vs ammonium) on the chlorophyll *a* (chl *a*), carbon (C), and nitrogen (N) quotas of the oceanic diatom *Actinocyclus* sp. was also examined. Under Fe-stressed conditions when the energy-stress on the cells is the greatest, ammonium-grown cells appeared to have a physiological advantage over nitrate-grown cells in this oceanic diatom. Ammonium-grown cells were able to maintain normal N and C quotas under Fe-stress, whereas nitrate-grown cells were not, resulting in an 80% reduction in N cell^{-1} for nitrate grown cells under Fe-stress. Also, *in vivo* fluorescence:chl *a* increased and chl *a*:C decreased more drastically for nitrate-grown cells under Fe-stress than for ammonium-grown cells, indicating that nitrate-grown cells under Fe-stress are less capable of trapping and utilizing light energy. These findings support theoretical predictions based on Fe and energy requirements for nitrate versus ammonium utilization. Metal quotas (Fe, Mn, Zn) were measured simultaneously using cold-metal techniques to determine the metal content of the cells. There were no significant differences in metal to carbon ratios between nitrate and ammonium-grown cells under Fe-replete conditions. Under Fe-stressed conditions, nitrate-grown cells had significantly higher Mn:C and significantly lower Zn:C ratios than ammonium-grown cells, but there was no observed difference in Fe quotas. In this study we observed that 2 different species of phytoplankton from the subarctic Pacific responded physiologically differently to similar Fe conditions. Our results suggest that the solitary, centric, 20 to 60 µm diameter oceanic diatom would have a higher sinking rate than the oceanic coccolithophore in the subarctic Pacific, perhaps having implications for biogenic fluxes to depth. Moreover, our data indicate that this diatom is probably utilizing ammonium to meet its nitrogen requirements *in situ* under the low Fe conditions found in the northeast subarctic Pacific. *Actinocyclus* sp. appears incapable of effectively changing its cell volume to help alleviate Fe- (and other nutrient) stress, whereas the coccolithophore can reduce its cell volume substantially, allowing it to reduce its requirements for N, C, and Fe. These physiological results help to explain phytoplankton composition dynamics in the subarctic Pacific.

KEY WORDS: Ammonium · *Emiliania huxleyi* · Iron · Metal quotas · Nitrate · Nitrogen source · Oceanic diatom · Subarctic Pacific · Sinking rate

INTRODUCTION

The subarctic Pacific is one of the 3 high nutrient low chlorophyll (HNLC) regions of the world's ocean where macronutrients (N, P, Si) are always in relatively high

concentrations, and low chlorophyll concentrations are found year round. Iron (Fe) is present in extremely low concentrations (<0.05 nmol kg^{-1}) and thought to limit phytoplankton growth rates (Martin et al. 1989) as well as controlling species composition in combination with grazing (Miller et al. 1991, Boyd et al. in press). Dominant phytoplankton species in the subarctic Pacific are

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typically small non-diatoms <5.0 µm, but diatoms are present and can, at times, be responsible for a substantial (30 to 50%) amount of the phytoplankton carbon (Booth 1981, 1988, Clemons & Miller 1984, Booth et al. 1988). As well, large pulses of diatoms do indeed sink to depth, with *Actinocyclus curvatulus* being a major species found in sediment traps in the spring (Takahashi 1986, Takahashi et al. 1990). It has been hypothesized that large diatoms are not consistently numerically abundant in the subarctic Pacific because their growth rates are limited by Fe, which prevents them from overcoming grazing pressure (Bruland et al. 1991, Morel et al. 1991, Price et al. 1991, Boyd unpubl.). It is further hypothesized that a lack of Fe prevents the utilization of nitrate (resulting in the ever-present supply of nitrate), which is assumed to be the favored nitrogen source of the Fe-limited cells once Fe is added (Martin et al. 1989).

Fe has many functions within a phytoplankton cell, including major roles in photosynthesis and respiratory electron transfer processes as well as nitrogen metabolism. It is well documented that Fe-stress will impair both the photosynthetic and respiratory pathways in algae and higher plants (Glover 1977, Spiller & Terry 1980, Terry 1980, 1983, Sandmann 1985, Greene et al. 1991, Greene et al. 1992), rendering the cell 'energy-stressed'. Large diatoms have been found to regulate their buoyancy by energy requiring processes, with energy derived from respiration as the principal source (Waite et al. 1992, Waite unpubl.). It follows then, that Fe-stress may affect phytoplankton sinking rates by stressing the energy producing pathways needed by the cell to maintain its buoyancy. In addition, the nitrogen source that a phytoplankter is grown on can affect the cell's growth rate and cell volume (Thompson et al. 1989, Levasseur et al. 1993), both of which may have indirect effects on sinking rates.

The nitrogen source that a cell uses can also have ramifications for the Fe requirements of a cell. Fe is required for the reduction of nitrate into amino acids, being a constituent of both nitrate and nitrite reductases (Goodwin & Mercer 1983). As ammonium does not require these reducing enzymes, the Fe requirement for ammonium utilization is less than that for nitrate utilization. It has been calculated that a cell grown on nitrate should theoretically require 60% more Fe than a cell grown on ammonium, given equal growth rates (Raven 1988). In addition, the nitrogen source that a cell uses can have ramifications for the energy status of that cell. For example, more energy is thought to be required for the transport of nitrate across the cell membrane than for ammonium (Falkowski 1975, Turpin & Bruce 1990). Also, more reductant is required for the assimilation of nitrate into amino acids compared to ammonium, with nitrate-grown cells requiring 22%

more reductant than cells growing on ammonium (Thompson et al. 1989). Since reductant is formed via photosynthetic or respiratory electron transport and Fe is a component of these systems, Fe-stress could result in less efficient energy transfer and possibly less production of reductant. Due to these differences in Fe and energy requirements depending on nitrogen source, it would be expected that a cell grown on ammonium would have an energy advantage over cells grown on nitrate. Under Fe-stress, cells grown on nitrate should theoretically be more severely 'stressed' than cells grown on ammonium. For example, nitrate-grown cells may not be able to maintain their normal nitrogen quotas under Fe-stress, whereas ammonium-grown cells may have normal nitrogen quotas. As well, the *in vivo* fluorescence relative to chlorophyll *a* (chl *a*) can be used as an indicator of the inefficiency of photosynthetic electron transfer (Lawlor 1987), and could theoretically be higher for nitrate-grown cells under Fe-stress, due to the additional Fe and energy requirements of utilizing nitrate.

We present here the first report on the effect of Fe on phytoplankton sinking rates, as well as reporting the effect of nitrogen source and Fe on the physiology and metal composition of a freshly isolated oceanic diatom, *Actinocyclus* sp., from the subarctic Pacific. The effect of Fe on the sinking rate of a small oceanic coccolithophore from the subarctic Pacific is also included as a comparison. Since cells under Fe-stress were examined in which their intracellular Fe concentration would be low, Mn, and Zn quotas were also measured to obtain basic metal composition data on this organism and to see if either metal increased under Fe-stress, possibly indicating a physiological use of the metal when Fe becomes limiting to the cell. Metal quotas were measured by cold-metal techniques to directly determine the metal content of the cells, allowing for the simultaneous measurements of multiple metals. Parameters measured include growth rate, cell volume, *in vivo* fluorescence, carbon quota, nitrogen quota, chl *a* quota, and metal quotas for Fe (intracellular and total), Mn, and Zn.

MATERIALS AND METHODS

The oceanic diatom *Actinocyclus* sp. was isolated in July 1993 from the subarctic Pacific Ocean (Stn Papa, 50° N, 145° W) by D. Muggli. Cultures were maintained in natural Stn Papa (P) water (with metals and nutrients added), in an attempt to retain the organism's native physiology and morphology.

Experiments were conducted with filter-sterilized, Chelex-treated (Price et al. 1988/89), Stn P water. Upon arrival from sea, Stn P water was filtered through an acid-cleaned 0.22 µm polycarbonate filter in a Teflon

filtration apparatus. A rigorous acid-cleaning procedure was used for all plastics coming in contact with the cultures and for all plastics used in the metal analysis as follows: 25% reagent grade (rg) HCl >24 h, 2 times rinse with Nanopure (or MilliQ) water, 0.01 M ultra HCl (Seastar Chemicals) >1 wk. The Stn P water was then passed through a Chelex 100 resin column, prepared as in Price et al. (1988/89), at a flow rate of <2 ml min^{-1} The water was then filter sterilized (0.22 µm) into a sterile, acid-cleaned polycarbonate carboy (sterilized by boiling Nanopure water). The total Fe in this chelexed Stn P water was measured as in Yang (1993) and found to be ca 0.9 nmol kg^{-1}. The same batch of water was used for all experiments.

Macronutrient enrichments were made with Chelex-treated stocks (see Price et al. 1988/89). The final concentrations in the medium were 150 µM Si, 5 µM PO_4, and 50 µM NO_3 or NH_4. Cells did not grow well with ammonium concentrations of 75 µM or higher. Metals were added to a final concentration of 1.5 times the AQUIL (artificial algal culturing medium) amounts as follows: 35 nM Mn, 12 nM Zn, 1.5 nM Cu, 150 nM Mo, 3.75 nM Co, 15 nM Se (Price et al. 1988/89). 10 µM EDTA was also added. ESAW amounts of vitamins were used (Harrison et al. 1980), and 1000 nM Fe final concentration was used for the Fe-replete treatment. The EDTA and Fe were added first and the medium was allowed to equilibrate for 48 h before use.

Triplicate cultures were grown in acid-cleaned 3 l polycarbonate Fernbach flasks equipped with Teflon tubing and Teflon stirring bars and handled only under class 100 flowhood conditions. The average irradiance was 145 µE m^{-2} s^{-1} as measured by a Biospherical Instruments model QSL-100 light meter. This irradiance was saturating for growth. Flasks were rotated once a day to ensure the same light conditions between flasks. Cultures were grown on a 14 h light:10 h dark cycle with Vitalite fluorescent bulbs at 16°C.

The cultures were maintained semi-continuously in either 'no Fe medium' (no Fe was added) or 'Fe-replete medium' (1000 nM Fe). These are referred to as the 'Fe-stressed' and 'Fe-replete' cells, respectively. Triplicate cultures of both nitrate and ammonium treatments were grown under both Fe conditions. Cultures were grown for over 10 generations before harvesting in log phase.

Cultures of Emiliania huxleyi, also isolated from Stn P, were grown on nitrate only in Chelex-treated (Price et al. 1988/89), microwave-sterilized (Keller et al. 1988), Stn P water 30 µM chelexed NO_3 and 2 µM chelexed PO_4 were added. For further details on culture conditions and how the cells were Fe-stressed see Muggli & Harrison (1996).

Growth rates were calculated from linear regressions of plots of ln cell number versus time. Cell num-

bers and cell volumes were measured on a Coulter Counter model TAII. Volumes were calculated using the equation for a cylinder, with the radius calculated from the Coulter Counter, and the height obtained by microscopic observation for Actinocyclus sp. Chl a concentrations were determined by in vitro fluorometry (Parsons et al. 1984). Samples for particulate organic carbon and nitrogen were collected on precombusted 13 µm Gelman A/E filters and analyzed on a Carlo Erba CNS analyzer. Sinking rates were measured by the SETCOL method (Bienfang 1981) in motionless 1 m Plexiglas columns over a 3 h period under the same irradiance conditions as the cultures. The SETCOL columns were acid-cleaned prior to measurement and filled in a class 100 trace metal free room for the Fe-stressed experiments.

Metal quotas were measured using cold-metal (non-radiotracer) techniques. All procedures were conducted under class 100 flowhood conditions. A known volume of culture was filtered onto acid-cleaned cellulose acetate 3.0 µm filters on a Nalgene polycarbonate filtration apparatus, either treated with Ti(III) or not to determine intracellular and total Fe, respectively (Hudson & Morel 1989), and was followed by a rinse with 10 ml of Chelex-treated Stn P water. Acid-cleaned cellulose acetate filters (5% rg HCl >24 h, 2 times rinse with Nanopure, 0.01 M ultra HCl >1 wk) gave comparable blanks to acid-cleaned polycarbonate filters (25% rg HCl >24 h, 2 times rinse with Nanopure, 0.01 M ultra HCl >1 wk) and were used for ease in digestion. To test for the possible adsorption of any contaminant Fe contained in the Ti(III) wash onto the cellulose acetate filters, filter blanks were also treated with the Ti(III) reagent and subtracted out from Ti(III) treated culture samples. The Ti(III) solution did not significantly increase the Fe content of the filter blanks, indicating that any Fe in the Ti(III) wash did not absorb onto the cellulose acetate filters or that the 10 ml rinse with Chelex-treated Stn P water rinsed off any contaminating Fe. The Ti(III) reagent was prepared fresh on the day of use, and no milky precipitate was ever observed. Three filter blanks were obtained for every culture treatment. Filters were digested in concentrated ultrapure HNO_3 (Seastar Chemicals) in screw-top Teflon jars for 24 h at room temperature. The digestate was diluted appropriately and metal concentrations were determined on a Varian graphite furnace atomic absorption spectrometer with Zeeman background correction. SLRS-2 riverine water (certified standard, Environment Canada) was used as a standard for accuracy. Ti(III) treated samples were also measured to determine both the intracellular and the intra- and extracellular (total) Fe content of the cells (Hudson & Morel 1989).

All statistical comparisons were made using a Student's t-test at the 95% confidence level ($p < 0.05$).

RESULTS

Sinking rate

Fe-stress had a dramatic effect on the sinking rate of the oceanic diatom (Fig 1A, Table 1). The sinking rate under Fe-stressed conditions was about 5 times greater than under Fe-replete conditions, with average sinking rates of 0.92 and 0.17 m d^{-1}, respectively. This is in sharp contrast to the results obtained with the oceanic coccolithophore, *Emiliania huxleyi* (Fig. 1B). Fe had no effect on the sinking rate of this small coccolithophore, with the rate under both Fe-stressed and Fe-replete conditions being about 0.12 m d^{-1}.

For *Actinocyclus* sp., the sinking rate for ammonium-grown cells under Fe-replete conditions was significantly greater than the sinking rate of nitrate-grown cells (Fig 1A, Table 1). There was no difference between the sinking rate of ammonium and nitrate-grown cells under Fe-stressed conditions.

Cell volume

The cell volume of *Actinocyclus* sp. decreased slightly but significantly from Fe-replete to Fe-stressed conditions (Fig. 2A, Table 1), with an 8 % decrease occurring for nitrate-grown cells and an 18 % reduction occurring for ammonium-grown cells. The cell volume of ammonium-grown cells under Fe-replete conditions was significantly greater than the cell volume of nitrate-grown cells under the same conditions. In contrast, the cell volume of *Emiliania huxleyi* decreased substantially when grown on nitrate, with a decrease of 46 % from Fe-replete to Fe-stressed conditions (Fig. 2B).
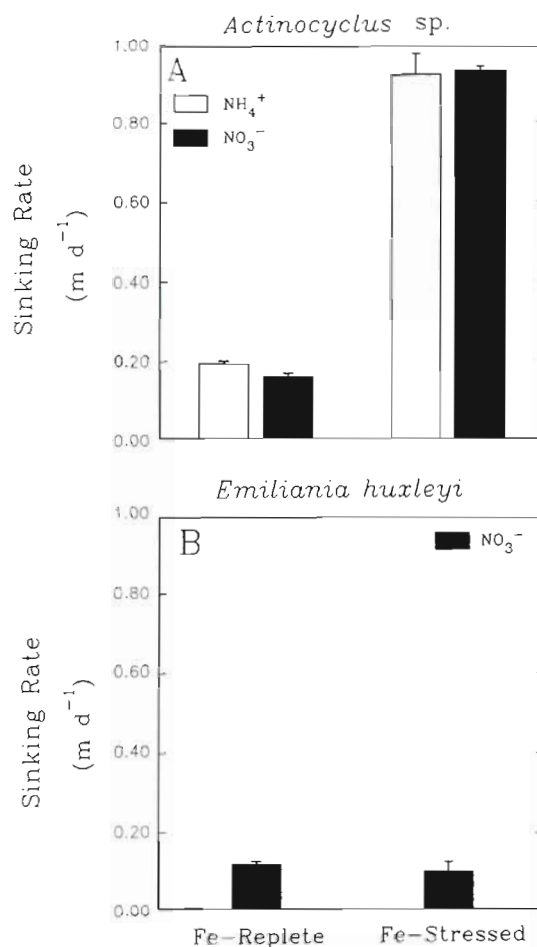

Fig. 1. *Actinocyclus* sp., *Emiliania huxleyi*. Sinking rate versus Fe condition for: (A) both ammonium and nitrate-grown cells of the centric oceanic diatom *Actinocyclus* sp., and (B) nitrate-grown cells of the oceanic coccolithophore *E. huxleyi*. Error bars represent SE (n = 3)

Table 1. *Actinocyclus* sp. Physiological parameters for nitrate and ammonium-grown cultures of the oceanic diatom *Actinocyclus* sp. under both Fe-replete and Fe-stressed conditions (SE in parenthesis; n = 3). $^*p < 0.05$ between NO_3 and NH_4-grown cells with the asterisk placed next to the greater value, $^{**}p < 0.05$ between Fe-replete and Fe-stressed cells with the asterisk placed next to the greater value. SR: sinking rate; CV: cell volume; (int): intracellular; (t): total

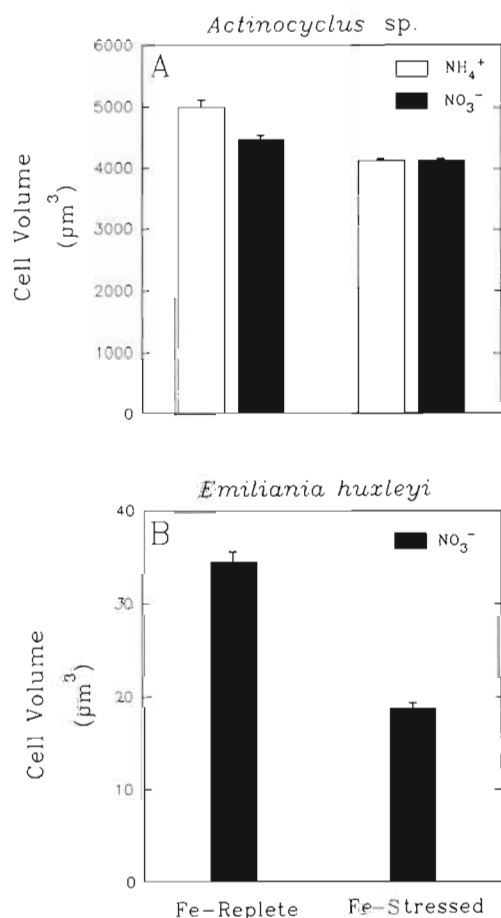| Parameter	Fe-replete				Fe-stressed			
	NH_4^+		NO_3^-		NH_4^+		NO_3^-	
SR (m d^{-1})	0.19*	(0.01)	0.16	(0.01)	0.92**	(0.05)	0.93**	(0.01)
CV (µm^3)	4990$^{*\cdots}$	(116)	4460**	(69)	4100	(26)	4100	(26)
Chl a:C (wt)	0.012**	(0.001)	0.022$^{*\cdots}$	(0.001)	0.006	(0.001)	0.009*	(0.001)
C:chl a (wt)	84*	(6)	45	(3)	167$^{*\cdots}$	(9)	106**	(10)
In vivo fl:chl a (ng chl a^{-1})	0.07*	(0.01)	0.06	(0.01)	0.12**	(0.01)	0.3$^{*\cdots}$	(0.02)
Chl a cell^{-1} (pg cell^{-1})	5.9**	(0.2)	8.7$^{*\cdots}$	(0.5)	3.1*	(0.1)	2.7	(0.2)
C cell^{-1} (pg cell^{-1})	496*	(39)	390**	(16)	519*	(26)	285	(1)
N cell^{-1} (pg cell^{-1})	82	(7.2)	70**	(2.3)	80*	(2.4)	38	(0.4)
C:N (mol:mol)	7.1	(0.5)	6.5	(0.1)	7.6	(0.3)	8.8$^{*\cdots}$	(0.1)
μ (h^{-1})	0.0174	(0.0022)	0.0192	(0.0017)	0.0109	(0.0015)	0.0149	(0.0006)
					0.0061	(0.0003)	0.0076	(0.0004)
Fe(int):C (µmol:mol)	330**	(85)	370**	(25)	66	(31)	38	(8)
Mn(t):C (µmol:mol)	45**	(3.3)	38	(2.7)	29	(0.2)	46*	(2.3)
Zn(t):C (µmol:mol)	3.4	(0.7)	5.8**	(1.0)	4.4*	(0.3)	1.6	(0.5)

Fig. 2. *Actinocyclus* sp. *Emiliania huxleyi.* Cell volume versus Fe condition for: (A) both ammonium and nitrate-grown cells of *Actinocyclus* sp.; and (B) nitrate-grown cells of *E. huxleyi.* Error bars represent SE (n = 3)

Chl *a* quotas

Under Fe-replete conditions, the chl *a* cell^{-1} was significantly higher for nitrate compared to ammonium-grown cells (Fig. 3A, Table 1). However, under Fe-stressed conditions, ammonium-grown cells had significantly higher chl *a* cell^{-1} than nitrate-grown cells (Fig. 3A, Table 1). Nitrate-grown cells decreased their chl *a* cell^{-1} by 70%, while ammonium-grown cells only decreased their chl *a* cell^{-1} by 40% under Fe-replete compared to Fe-stressed conditions.

The chl *a*:C ratio was significantly higher for Fe-replete cells than for Fe-stressed cells of *Actinocyclus* sp., with ammonium-grown cells showing a 50% reduction and nitrate-grown cells showing a 60% reduction in chl *a*:C under Fe-stressed conditions (Table 1). Nitrate-grown cells had greater chl *a*:C ratios than ammonium-grown cells under both Fe treatments.

In vivo fluorescence:chl *a*

The *in vivo* fluorescence (fl):chl *a* ratio was significantly higher under Fe-stressed conditions for both nitrate and ammonium-grown cultures of *Actinocyclus* sp. (Fig. 3B). However, *in vivo* fl:chl *a* increased 80% for nitrate-grown cells, but only 40% for ammonium-grown cells.

Carbon

The carbon content decreased significantly between Fe-replete and Fe-stressed cells of *Actinocyclus* sp. grown on nitrate (Fig. 4A, Table 1). However, the carbon content per cell remained constant for ammonium-grown cells under both Fe conditions. The carbon content was significantly greater for ammonium-grown cells than for nitrate-grown cells under both Fe treatments.
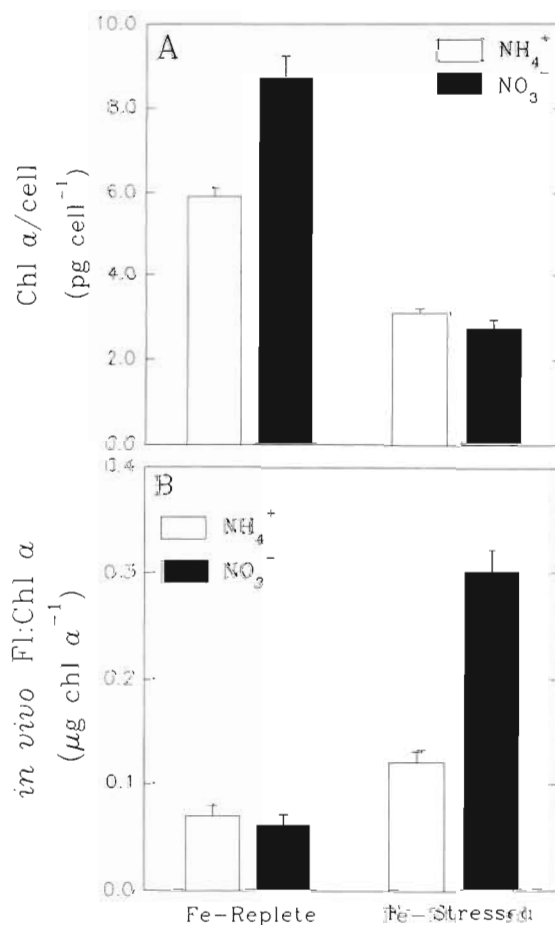

Fig. 3. *Actinocyclus* sp. (A) Chl *a* per cell and (B) *in vivo* fluorescence per chl *a* versus Fe condition for both ammonium and nitrate-grown cells of *Actinocyclus* sp. Error bars represent SE (n = 3)
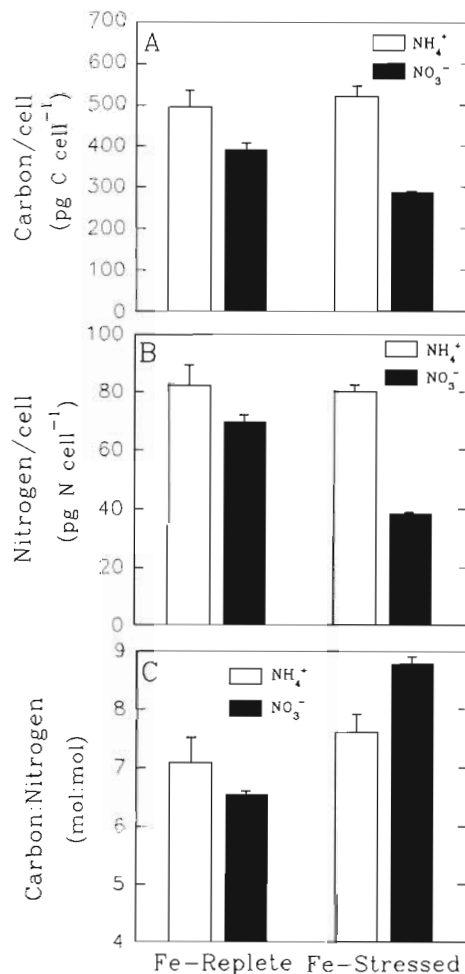
Fig. 4. (A) *Actinocyclus* sp. Carbon cell^{-1}; (B) nitrogen cell^{-1}; and (C) carbon:nitrogen ratio versus Fe condition for both ammonium and nitrate-grown cells of the oceanic diatom. Error bars represent SE (n = 3)
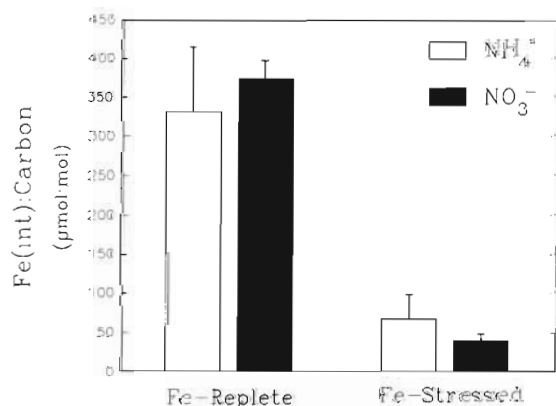

Fig. 5. *Actinocyclus* sp. Intracellular Fe:carbon ratio versus Fe condition for both ammonium and nitrate-grown cells of the oceanic diatom from the subarctic Pacific. Error bars represent SE (n = 3)

Nitrogen

Similar to carbon, the nitrogen content per cell decreased significantly for nitrate-grown cells, but not for ammonium-grown cells of *Actinocyclus* sp., under Fe-stressed conditions (Fig. 4B, Table 1). The nitrogen content decreased by about 80% for nitrate-grown cells. Again, ammonium-grown cells had more N cell^{-1} than nitrate-grown cells under both Fe treatments.

Carbon:nitrogen

The C:N ratio increased significantly for nitrate-grown cells of *Actinocyclus* sp. from Fe-replete to Fe-stressed conditions (Fig. 4C, Table 1). C:N values of ammonium-grown cells remained constant.

Metal quotas

Iron. The intracellular Fe to cellular carbon ratios, [Fe(int):C], decreased significantly between Fe-replete and Fe-stressed treatments, with about an 80% reduction in this ratio (Fig. 5, Table 1). There was no significant difference between nitrate and ammonium-grown cells for either Fe treatment.

Manganese. Mn:C ratios were not significantly different between nitrate and ammonium-grown cells under Fe-replete conditions (Table 1). However, under Fe-stressed conditions, nitrate-grown cells had significantly higher Mn:C ratios than ammonium-grown cells (Table 1). Ammonium-grown cells had significantly greater Mn:C ratios under Fe-replete versus Fe-stressed conditions; nitrate-grown cells had greater Mn:C ratios under Fe-stressed compared to Fe-replete conditions, although not significant at p < 0.05 (p < 0.10) (Table 1).

Zinc. There was no difference in Zn:C ratios between nitrate and ammonium-grown cells under Fe-replete conditions (Table 1). Under Fe-stressed conditions, ammonium-grown cells had higher Zn:C ratios than nitrate-grown cells (Table 1). Fe-replete cells had higher Zn:C ratios than Fe-stressed cells for nitrate-grown cultures (Table 1).

Effect of Ti(III) treatment

The effect of the Ti(III) treatment on Fe, Mn, and Zn quotas is illustrated in Fig. 6. The Ti(III) wash significantly removed extracellular Fe for both nitrate and ammonium-grown cultures under Fe-replete conditions (Fig. 6A). However, when particulate Fe values were low (as in the Fe-stressed cells), the Ti(III) reagent did not statistically alter the Fe quotas at

p < 0.05. As well, the Ti(III) treatment did not significantly alter the Mn content per carbon regardless of conditions (Fig. 6B). Under one circumstance, the use of the Ti(III) wash significantly increased the amount of Zn retained on the filters (Fig. 6C)

DISCUSSION

This is the first report on any physiology of an oceanic diatom isolated from the NE subarctic Pacific. As the subarctic Pacific is an HNLC region where Fe plays a key role in the physiology of the indigenous phytoplankton, it is important that we study the oceanic species from this region if we are to understand how Fe may affect the dynamics of phytoplankton assemblages in this region. As pointed out by Wells et al. (1995), published reports of laboratory studies on species from field sites in question (e.g. HNLC regions) are completely absent. Furthermore, this is the first study on the possible effects of Fe on the sinking rate of any type of phytoplankton.

Fe and sinking rate

Large oceanic diatom vs small oceanic coccolithophore

The sinking rate of the oceanic diatom *Actinocyclus* sp. was drastically affected by the Fe status of the cells, whereas the sinking rate of the coccolithophore *Emiliania huxleyi* was not affected by its Fe status (Fig. 1). This differing effect of Fe status on sinking rate suggests that the relatively large diatom (20 μm diameter) may depend on energy requiring processes to maintain its buoyancy, while the small coccolithophore (5 μm diameter) does not. This supports the trend observed by A. M. Waite, A. Fisher, P. A. Thompson & P. J. Harrison (unpubl.), namely, that larger cells tend to actively control their sinking rates by energy requiring processes, whereas small cells do not. The fact that Fe-stress was able to induce such a large increase in the sinking rate of *Actinocyclus* sp. is another piece of evidence indicating that energy production from electron transport systems may somehow be involved in the maintenance of buoyancy of diatoms (Waite et al. 1992). The sinking rate measured for *Actinocyclus* sp. under Fe-replete conditions is similar to the sinking rate maintained by *E. huxleyi*, which would also indicate that the large diatom is somehow actively reducing its sinking rate to levels lower than would be expected based on the size of the cell.
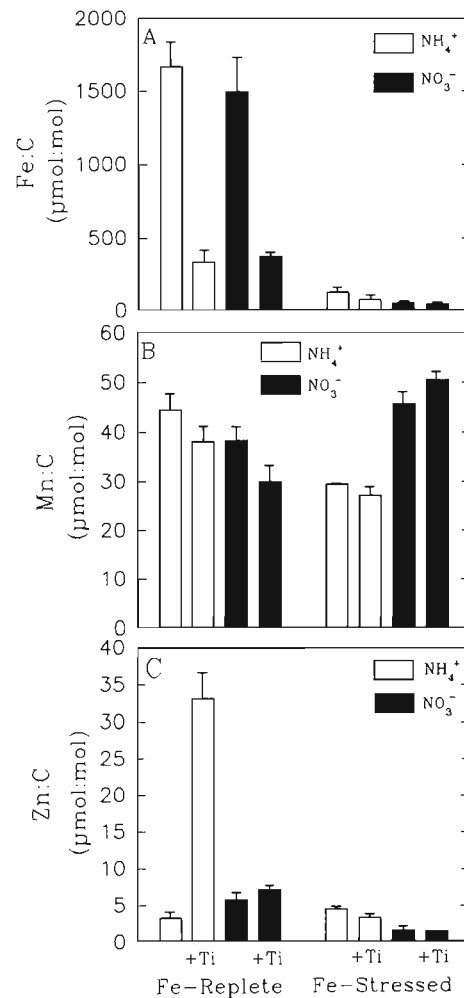

Fig. 6. *Actinocyclus* sp. (A) Fe:carbon; (B) Mn:carbon; and (C) Zn:carbon versus Fe condition for both ammonium and nitrate-grown cells of the oceanic diatom from the subarctic Pacific. Error bars represent SE (n = 3). '+Ti' indicates the Ti(III) reagent was used (see 'Materials and methods')

Sinking rate of NH_4 vs NO_3-grown cells of an oceanic diatom

Under Fe-replete conditions, the sinking rate of ammonium-grown cells was greater than the sinking rate of nitrate-grown cells of *Actinocyclus* sp. This could be due to the larger size of the ammonium-grown cells. However, the difference in sinking rate between ammonium and nitrate-grown cells was small compared to the observed difference between Fe-replete and Fe-stressed cultures. There was no difference between the sinking rate or cell volume of ammonium and nitrate-grown cells of *Actinocyclus* sp. under Fe-stressed conditions. Because of the extra severity of energy stress upon nitrate-grown cells, the nitrate-grown cells might have been

expected to have higher sinking rates. It is possible that the effect of Fe on sinking rate is a 'step response', where a threshold is reached above which the cells have no physiological control of their sinking rate. Then, due to their identical cell volume, the ammonium and nitrate-grown cells would be expected to sink at the same rate. The only way to test this possibility is to measure sinking rates under differing degrees of Fe-stress.

Cell volume, Fe, and sinking rates

The change in cell volume observed for the diatom cannot explain the change in sinking rate observed between Fe-replete and Fe-stressed conditions. In fact, a decrease in the cell volume of Fe-stressed cells should produce a decrease in sinking rate. Therefore, the 5 times increase in sinking rate under Fe-stress would have been even higher if it were not for the small decrease in cell volume under Fe-stress.

The decrease in cell volume of *Actinocyclus* sp. can be solely attributed to the natural decrease by asexual cell division (Fig. 7). The cell volume of *Actinocyclus* sp. for Fe-replete cultures grown on both nitrate and ammonium was monitored for 40 d, which was longer than the experiments reported here (25 d). A linear regression was used to predict the decrease in the cell volume expected during asexual division for the duration of the experiments. The cell volumes measured for the Fe-stressed cultures lie above the predicted cell volumes calculated using the regression equations and the initial cell volumes of the cultures.

In contrast to the diatom, the cell volume of *Emiliania huxleyi* grown on nitrate decreased drastically under

Fe-stress (46%), much lower than could be explained by normal daily variations in its cell volume. It is interesting to note, however, that *E. huxleyi* growing on ammonium did not decrease its cell volume under Fe-stress (Muggli & Harrison 1996). It appears that *E. huxleyi* can reduce its cell volume when growing on nitrate under low Fe conditions, helping it to maintain its low sinking rate, whereas the diatom cannot change its cell volume enough to compensate for the very large increase in sinking rate under Fe-stressed conditions. Reducing its cell volume is one way in which *E. huxleyi* may be able to adapt to its low Fe environment in the subarctic Pacific. A reduction in cell volume is particularly advantageous for cells living in a low Fe environment because it not only reduces sinking rates, but it reduces cellular requirements for N, C, and Fe (Muggli & Harrison 1996). Therefore, the larger diatom, which is unable to make large changes in its cell volume, may be more susceptible to Fe-stress in the subarctic Pacific than small cells that can reduce their cell volume and become even smaller.

Fe and chlorophyll, carbon, nitrogen

The chl a:C ratio was lower for Fe-stressed cells, presumably due to the fact that Fe is required in the synthesis of chlorophyll (Spiller et al. 1982). This is in agreement with other researchers who found reductions of chl a quotas under Fe-stress for diatoms (Glover 1977, Greene et al. 1991, Greene et al. 1992), a dinoflagellate (Doucette & Harrison 1990), cyanobacteria (Gikema & Sherman 1983, Rueter et al. 1990, Rueter & Unsworth 1991), a chlorophyte (Greene et al. 1992), and a freshwater green algae (Rueter & Ades 1987). The oceanic coccolithophore *Emiliania huxleyi* is an exception, since it does not reduce its chl a quota under Fe-stressed conditions limiting to growth (Muggli & Harrison 1996). The fact that nitrate-grown cells had higher chl a:C values than ammonium-grown cells is due to the lower C cell^{-1} of the nitrate-grown cells, as the chl a cell^{-1} was significantly lower for nitrate-grown cells compared to ammonium-grown cells under Fe-stress (Fig. 3A, Table 1).

The *in vivo* fl:chl a ratio was found to be higher under Fe-stress, similar to other studies (Sakshaug & Holm-Hansen 1977, Gikema & Sherman 1983, Doucette & Harrison 1990, Doucette & Harrison 1991). However, the nitrate-grown cells exhibited an 80% increase in this ratio (compared to 40% for ammonium-grown cells), indicating that the nitrate-grown cells had less efficient photosynthetic electron transfer processes occurring than the ammonium-grown cells. Nitrate-grown cells of a dinoflagellate also exhibited higher *in vivo* fl:chl a compared to ammonium-grown cells
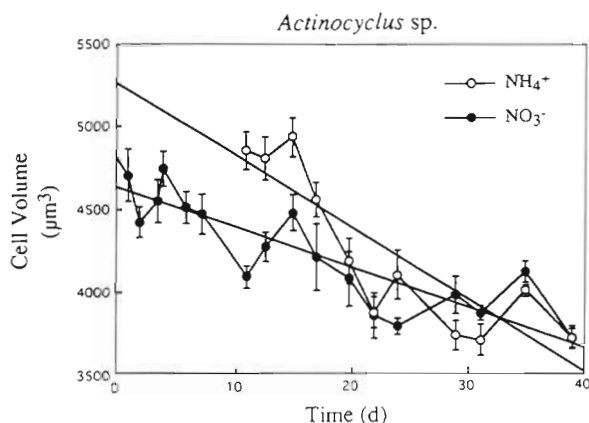

Fig. 7. *Actinocyclus* sp. Cell volume versus time for both ammonium and nitrate-grown cultures of the oceanic diatom grown in Fe-replete medium over a 40 d period. Error bars represent SE (n = 3). The slope of the linear regressions are −24.7 (r^2 = 0.8) and −45.2 (r^2 = 0.8) for nitrate and ammonium-grown cultures, respectively

under Fe-stress (Doucette & Harrison 1991). Nitrogen limitation can also increase in vivo fl:chl a (Kiefer 1973, Sakshaug & Holm-Hansen 1977), as nitrogen is required as well as Fe for the synthesis of cytochromes (Pushnik et al. 1984). The nitrate concentration in the medium at the time of harvesting the Fe-stressed cells was >30 µM nitrate (>10 µM ammonium for ammonium-grown cultures). However, nitrate uptake rates were not measured, and it is possible that the nitrate-grown cells were partially 'N-stressed' as well, due to inability of nitrate-grown cells under energy restraints (i.e. Fe-stress) to transport and assimilate nitrate. The N cell^{-1} value remained the same for ammonium-grown cells, but decreased significantly for nitrate-grown cells under Fe-stress (Table 1), indicating that the nitrate-grown cells were not able to maintain their optimal nitrogen quotas. This was also found to occur in the red tide dinoflagellate Gymnodinium sanguineum, with ammonium-grown cells containing 50% more N CV^{-1} than nitrate-grown cells (Doucette & Harrison 1991). This is in agreement with theoretical expectations, and is reflected in the increased C:N ratio for nitrate-grown cells under Fe-stress, despite a significant decrease in C quota.

The greater reduction in chl a cell^{-1}, the greater increase in in vivo Fl:chl a, and the lower N cell^{-1} for nitrate-grown cells compared to ammonium-grown cells under Fe-stressed conditions all support the theoretical predictions that cells utilizing nitrate should be more Fe-stressed that cells utilizing ammonium.

Fe quotas

The intracellular Fe to cellular C ratio of the cells decreased ca 80% from Fe-replete to Fe-stressed conditions in this experiment. However, there was no difference in the Fe(int):C ratios between nitrate and ammonium-grown cultures for either Fe treatment (Fig. 5). The C cell^{-1} was lower for the nitrate-grown cells, and it is possible that the decrease in C resulting from energy constraints on the cells cancels out any necessary increase in Fe content. Based on theoretical calculations (Raven 1988), nitrate-grown cells should contain more Fe that ammonium-grown cells (1.7 times), but this comparison can only be made if the cells are growing at identical growth rates (not the case in our study) and probably only under replete energy (Fe) conditions.

Other researchers have measured cellular Fe to cellular C ratios in phytoplankton, but by radiotracer techniques. Measuring metal quotas directly by cold-metal techniques allows for the determination of multiple metals simultaneously. However, limitations exist, especially when attempting to measure intracellular

metals only. The Ti(III) treatment was developed for use with radioisotopes of Fe; the method should not be used for other metals nor when particulate Fe values are extremely low. This technique should also not be used when working with small pore-sized filters, as the longer filtration time required may allow significant Ti(III) oxidation to occur, resulting in precipitation on the filter (R. Hudson pers. comm.). When cells are Fe-stressed, they may increase the number of Fe uptake sites on their cellular membranes, thereby increasing the likelihood of any Fe in the Ti(III) solution binding to the cell surface. In these experiments Ti(III) treated cells always resulted in lower Fe values than non-Ti(III) treated cells, with the internal values of Fe-stressed cells being ≤75% of the total Fe values, in agreement with previous experiments of Fe-stressed cells of a diatom using radiotracer techniques (Hudson & Morel 1990). As well, our Fe-stressed cells were not severely stressed, as cell division was still taking place. However, the possibility exists that some binding of contaminant Fe in the Ti(III) wash may exist, resulting in slightly elevated intracellular Fe quotas for the Fe-stressed cells. Again, we found no adsorption of Fe onto the cellulose acetate filters, but binding to Fe uptake sites on the cells may take place, with not all of this Fe being removed from the Ti(III) treatment.

We can compare the Fe requirements for growth of our diatom from the subarctic Pacific with other phytoplankton species. The relationship between Fe(int):C with growth will be presented elsewhere (Muggli & Harrison unpubl.). In general, cyanobacteria appear to have the highest Fe requirements for growth of any phytoplankton species, with marine and freshwater cyanobacteria ranging from 90 to 600 µmol Fe:mol C to maintain 90% of maximal growth (Rueter et al. 1990, Hutchins et al. 1991, Rueter et al. 1992). Only a single dinoflagellate has been examined to date, and the minimum Fe required for cell division to occur was found to be 110 µmol Fe:mol C (Doucette & Harrison 1991). If this one dinoflagellate species (Gymnodinium sanguinium) is indicative of all dinoflagellate species, both the oceanic coccolithophore and diatom from the subarctic Pacific have far lower Fe requirements for growth. Diatom species tend to be the organisms used most frequently in phytoplankton/trace-metal studies, with nearly all data obtained from Thalassiosira spp. The 2 coastal species studied, T. weissflogii and T. pseudonana, are reported to require 5 to 8 µmol Fe:mol C to maintain 90% of maximal growth (Harrison & Morel 1986, Sunda et al. 1991). An oceanic Thalassiosira sp., T. oceanica, has been reported to have the lowest Fe requirement for growth thus far, requiring only 2 µmol Fe:mol C for 90% of maximal growth (Sunda et al. 1991). An indication of error is not given in the estimates of these ratios. As well, large amounts

of EDTA (100 μM) were used in Fe-stressing the oceanic diatom, which we have found to alter the physiology of our oceanic isolates (Muggli & Harrison unpubl.).

From the relationship of Fe(int):C versus growth for *Actinocyclus* sp. (Muggli & Harrison unpubl.), the minimum Fe:C ratio required for cell division to occur is 15 μmol Fe:mol C, and the Fe:C ratio required for 90% of maximal growth is 76 μmol Fe:mol C. These requirements appear high relative to other reported Fe requirements for growth of diatoms. However, Martin et al. (1989) suggested a ratio of 30 μmol Fe:mol C as representative of biogenic particles in the northeast Pacific, indicating that our estimates for this diatom from the northeast subarctic Pacific are reasonable. Why a species from a low Fe environment would have a higher Fe requirement than species from coastal habitats in unclear, although diatoms in the subarctic Pacific most likely grow only when additional Fe is added to the system. *Actinocyclus* sp. will not divide in unaltered Stn P water with no chelators present, while the oceanic coccolithophore *E. huxleyi* will grown maximally (Muggli & Harrison unpubl.). Hence, this diatom is a high Fe-requiring species, even for this region of the ocean.

Mn and Zn quotas

Under Fe-replete conditions, there was no difference in Mn or Zn quotas with nitrogen source. Under Fe-stressed conditions, nitrate-grown cells had significantly higher Mn quotas than ammonium-grown cells (Table 1). This may indicate a greater requirement for Mn under Fe-stressed conditions, as the nitrate-grown cells showed greater signs of Fe-stress than the ammonium-grown cells. As well, nitrate-grown cells had higher Mn quotas under Fe-stress than under Fe-replete conditions at a significance level of $p < 0.10$. An increase in Mn quota under Fe-stress has also been found by other researchers; Harrison & Morel (1986) observed the same phenomenon while working with a coastal diatom. It appears that this response is not solely limited to diatoms, as increased Mn quotas under Fe-stressed conditions have also been found in an oceanic coccolithophore, *Emiliania huxleyi*, when grown on nitrate (Muggli & Harrison 1996). Harrison & Morel (1986) suggested that Mn uptake may be inhibited at high Fe concentrations. There could also be a real, physiological requirement for Mn under low Fe conditions that has not yet been identified.

The Zn:C ratio was higher for ammonium-grown cells under Fe-stressed conditions; the opposite of what was found for Mn. No reports of Zn quotas under different nitrogen sources have been made previously. Since the Zn quota for nitrate-grown cells under Fe-

stress were significantly lower than ammonium-grown Fe-stressed cells and Fe-replete nitrate-grown cells, the transport of Zn into the cell may be impaired under energy-stressed conditions, with nitrate-grown cells being more adversely affected than ammonium-grown cells.

Values for Mn and Zn quotas obtained from radio-tracer methods have been previously reported in the literature for 2 diatom species (both *Thalassiosira* sp., one from the Sargasso Sea), and for 3 diatom species (all *Thalassiosira* sp., one from the Sargasso Sea), respectively (Sunda & Huntsman 1983, 1992). For Mn:C, the values range from 22 to 32 μmol Mn:mol C as reported in Sunda (1988/89). *Actinocyclus* sp. values ranged from 30 to 45 μmol Mn:mol C under all conditions tested, lying in the upper range of previously reported values. The Zn:C ratios (3 and 7 μmol Zn:mol C) for *Actinocyclus* sp. under Fe-replete conditions with EDTA present are lower than previous reports for diatoms, which range from 11 to 14 μmol Zn:mol C (Sunda & Huntsman 1992).

Ecological significance

Fe status of the cell had an effect on the sinking rate of an oceanic diatom, but not on an oceanic coccolithophore from Stn P. Since the major source of Fe in the subarctic Pacific is thought to be aeolean (Martin et al. 1989), the low sinking rate of the coccolithophore may give it an advantage if Fe was in fact limiting the metabolism of the indigenous phytoplankton. However, the sinking rate of Fe-stressed cells of this diatom was <1 m d^{-1}, which is probably not fast enough to result in sinking to depth. This sinking rate is high for a physiologically active diatom cell, but most likely some other mechanism must be invoked for the occurrence of large fluxes of siliceous material to sink to depth. Clumping is one way in which cells can sink to depth with greater speed, but no such phenomenon was observed in our cultures. However, cells were never allowed to go into senescence during our experiments, and stationary, unhealthy cells are more likely to clump and stick together. Smetacek (1985) proposed that once cells become severely stressed aggregates may form, scavenging other minerals as well, drastically accelerating sinking rates of the new particles (≥ 100 m d^{-1}). Therefore, an increase in the sinking rate of a physiologically active cell may be an indicator of other processes about to occur if that cell is not able to return to a healthier state in a given amount of time. The time frame involved for senescent cells to recover or actually lyse is largely unknown and very variable between species (J. Berges pers. comm.). We know that large pulses of siliceous material do indeed sink to

depth (3800 m) at Stn P because of sediment trap data (C. S. Wong unpubl. data), and in fact, *Actinocyclus curvatulus* is a dominant component of this material, exhibiting the greatest flux in the spring (Takahashi 1986, Takahashi et al. 1990). However, the exact mechanism resulting in this flux remains a mystery.

Since the sinking rate of the diatom is high under 'Fe-stressed' conditions (0.9 m d^{-1}), this diatom would probably exist in a lower light environment. It has been found that Fe-deficient cells may be more susceptible to photochemical damage than Fe-replete cells (Geider & LaRoche 1994), implying that Fe-stressed cells may be better off in low light. As well, the lower light may allow the cell's metabolism to remain slow, decreasing its requirements for photosynthate and nutrients. However, low light is also theoretically expected to increase the Fe requirements of a cell (Raven 1988, 1990), although this has yet to be demonstrated in the laboratory. Any interactions between irradiance, Fe, and nitrogen source were not examined in this study. More detailed laboratory studies on the combined effects of irradiance and Fe-stress on sinking rates, along with examining senescent-phase cultures are needed to further understand the role of Fe in causing fluxes of phytoplankton material to depth.

From these experiments, it would seem to be more advantageous for *Actinocyclus* sp. to utilize ammonium for its nitrogen requirements under low Fe conditions, as nitrate-grown cells exhibited greater signs of physiological stress than ammonium-grown cells under the Fe-stressed conditions tested (lower chl *a* cell^{-1}, higher *in vivo* fl:chl *a*, lower N and C cell^{-1}). This is in agreement with theoretical predictions made regarding nitrogen and Fe, as well as supporting field hypothesis. Price et al. (1991) found that the indigenous phytoplankton from the equatorial Pacific, another HNLC region, were indeed utilizing ammonium as their primary nitrogen source. This was a mixed assemblage, but some diatoms were present. Assessing which nitrogen source diatoms exclusively are utilizing in the field is difficult, as it is impossible to separate out only diatoms in the field, and the larger diatoms are not numerically abundant at Stn P. However, nitrogen uptake measurements have been made at Stn P for nitrate, ammonium, and urea, and samples were size-fractionated, with the largest size (>18 μm) being predominantly diatoms (D. Varela unpubl. data). Results from these measurements are in preparation. Since ambient nitrate concentrations are never depleted at Stn P and ammonium is present at low concentrations but supplied constantly (0.2 to 0.6 μM within the euphotic zone; D. Varela pers. comm.), it is possible that this diatom is utilizing ammonium to meets its nitrogen requirements for at least part of the time. Given the addition of Fe via atmospheric deposi-

tion (Martin et al. 1989), the only physiological evidence we found indicating that cells utilizing nitrate may be better off than cells utilizing ammonium, was that nitrate-grown cells had higher chl *a* cell^{-1} and lower *in vivo* fl:chl *a* than ammonium-grown cells. This could indicate that nitrate-grown cells may be photosynthesizing faster or more efficiently, although it did not result in a significantly faster growth rate in our experiments. In order to determine if Fe-stressed cells given Fe switch from utilizing ammonium to nitrate, the measurement of ammonium and nitrate uptake rates in the presence of both nitrogen sources at ecologically relevant concentrations is necessary. Our data do not refute this possibility. Field experiments have indirectly supported the hypothesis that diatoms 'switch' from ammonium to nitrate utilization when Fe is added to natural Stn P water (Martin et al. 1989, Boyd et al. in press). Experiments conducted in field conditions with enclosures can not distinguish whether the draw down of nitrate upon addition of Fe is due to more cells growing up (caused by an alleviation of Fe-stressed growth rates, or the elimination of grazers), or a switch from ammonium to nitrate utilization, as previously mentioned by Price et al. (1991). Again, the best way to address this physiological question is via controlled laboratory experiments. Ideally, Stn P water with no artificial chelators would be used (see Muggli & Harrison unpubl.).

Our study gives a physiological basis as to why an oceanic diatom from the subarctic Pacific should utilize ammonium rather than nitrate for its nitrogen requirements under low Fe conditions, as well as providing basic physiological and metal composition parameters of *Actinocyclus* sp. Large diatom species flux to depth at Stn P (Takahashi 1986, Takahashi et al. 1990), and studying the ecophysiology of large diatoms may help to understand the ecological dynamics occurring in the subarctic Pacific.

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