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Fulfilling iron requirements of a coastal diatom under different temperatures and irradiances

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Abstract

The strategies used by a coastal diatom, *Thalassiosira pseudonana*, for potentially different iron (Fe) requirements under different temperatures and irradiances were examined on the basis of three parameters: Fe uptake rate, cell-specific growth rate, and Fe efflux rate constant. These three variables determined the cellular Fe concentration, and they were all quantified under different temperatures and irradiances during long-term (days) and short-term (hours) ^{59}Fe exposures. Results obtained from both exposures were consistent. Although more Fe was required under the lower irradiance, Fe uptake rate decreased 1.78× and 2.20× as the irradiance decreased from 340 to 40 μmol photons m^{-2} s^{-1} when measured by short- and long-term exposures, respectively. Under this condition, the cell-specific growth rate decreased from 1.30–1.50 to 0.51–0.63 d^{-1} to keep a relatively high intracellular Fe concentration under lower irradiance. The opposite trend was observed for temperature. The higher Fe requirement at higher temperature was fulfilled mainly through an increase of Fe uptake rate with increasing temperature. For example, the Fe uptake rate increased by 1.21× and 2.55× as the temperature increased from 15°C to 24°C in the short- and long-term exposures, respectively. In contrast, the cell-specific growth rate was relatively constant (0.92–1.06 d^{-1}) under these three temperatures. Fe efflux from the diatoms was significant, with an efflux rate constant ranging from 0.008 to 0.017 h^{-1}. Such a high Fe efflux suggested that it should not be neglected in the calculation of intracellular Fe concentration. Furthermore, Fe efflux might be an important process for Fe regeneration in surface seawater. However, Fe efflux had a negligible effect on the different Fe requirements under different temperatures and irradiances.

Iron (Fe) is an essential trace metal in marine phytoplankton. It is involved in many biochemical processes, such as photosynthetic and respiratory electron transport, pigment synthesis, nitrate and nitrite reduction, and other numerous biochemical reactions (Geider and La Roche 1994). Its bioaccumulation mechanism by phytoplankton has been examined (Hudson and Morel 1990; Sunda and Huntsman 1995; Maldonado and Price 1999). To quantify Fe accumulation in marine phytoplankton, both long- and short-term exposures have been widely used (Sunda and Huntsman 1995; Kuma et al. 2000; Wang and Dei 2001). In the long-term exposure experiments, Fe accumulation is generally quantified at the end of the exposure with a steady-state assumption. The ambient metal concentration was kept constant during this period with the help of high concentrations of buffer reagents (e.g., edatic acid [EDTA]). This method is relatively simple, with only one time point, but the metal uptake kinetics cannot be obtained nor can cell surface adsorption, which is completed within a few minutes (Hassler et al. 2004). The cells can also excrete some metabolites during the long-term exposure period; thus, the water chemistry and metal speciation might be affected, which are critical in metal accumulation (Wilhelm et al. 1996; Marañón et al. 2004; Wei and Ahner 2005). In the short-term exposure experiments, cellular metal accumulation is quantified at different time points, lasting a few minutes to hours. The uptake kinetics and cell surface adsorption can be measured by this method, and the potential effect of organisms on the ambient environment can be minimized. Although these two methods have both been applied frequently in metal accumulation studies, no direct comparison of both approaches has been made in previous studies.

Both temperature and light are important environmental variables in the ocean. Raven (1990) calculated that more Fe was needed in the phytoplankton at lower irradiance for the much higher pigment synthesis to intercept more light energy. It has also been found that phytoplankton growth at high temperature mimics low-light acclimation (Maxwell et al. 1994) and thus requires more Fe (Davison 1991). Under steady-state conditions the cellular Fe concentration can be expressed with Eq. 1.

$$C_{ss} = \frac{V_{ss}}{\mu + k_e} \quad (1)$$

C_{ss} and V_{ss} represent the cellular Fe concentration and Fe uptake rate under steady-state condition, respectively; μ is the cell-specific growth rate; and k_e is the Fe efflux rate constant from the cell. According to this equation, cellular Fe concentration is determined by three variables (i.e., V_{ss}, μ, and k_e). It is thus interesting to examine how phytoplankton fulfill their different cellular Fe requirements under different light and temperature conditions on the basis of these three variables.

The Fe uptake (i.e., V_{ss}) under the influence of environmental factors, such as light and temperature, has not been well studied. Both light and temperature can directly affect Fe chemistry in seawater as well as phytoplankton growth

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and cellular composition. Their net effects on Fe accumulation in marine phytoplankton and further Fe biogeochemical cycling are rather speculative. In several studies, no consistent results were found about the influences of temperature and light on Fe uptake. For example, the Fe uptake rate can either increase, remain constant, or even decrease with an increase in irradiance (Sunda and Huntsman 1997; Schmidt and Hutchins 1999; Strzepek and Price 2000). Meanwhile, direct quantification of Fe efflux in marine phytoplankton has not yet been performed. It remains unknown whether temperature and light intensity have any effects on Fe efflux. The potential existence of Fe efflux can not only affect intracellular Fe concentration (as indicated in Eq. 1) but also provide an important Fe source for the growth of marine phytoplankton because dissolved Fe concentration is exceptionally low in surface waters (e.g., high-nitrate, low-chlorophyll area). The input of regenerated Fe is estimated to be orders of magnitude greater than the external supply rate of iron (Wells et al. 1995). Hutchins et al. (1993) found evidence of the potential biological recycling of Fe between cyanobacteria and diatoms, and it remains to be determined whether Fe efflux plays a role in such potential biological recycling.

To know how phytoplankton fulfill their different Fe requirements under different temperatures and irradiances, we conducted both long- and short-term uptake experiments to examine their influences on Fe uptake by a coastal diatom, *Thalassiosira pseudonana*. Cell-specific growth rates under the different light and temperature conditions were also quantified. We further measured the intracellular Fe efflux from diatoms, as well as its dissociation from the cell surface under each condition. The diel cycle of Fe uptake by the diatoms was also examined because the Fe requirement by phytoplankton might vary diurnally (Falkowski and La Roche 1991).

Material and methods

An axenic culture of *T. pseudonana* (CCMP 1335, clone 3H) was obtained from the Provasoli–Guillard Center for the Culture of Marine Phytoplankton, Bigelow Laboratory, West Boothbay Harbor, Maine. The cells were maintained in f/2 medium (Guillard and Ryther 1962) under sterile conditions in an incubator at 19°C with a light illumination of 170 μmol photons m^{-2} s^{-1} on a 14 : 10 h light : dark (LD) cycle. All seawater was collected 10 km off East Hong Kong to minimize the influences of anthropogenic activities. It was filtered through a 0.22-μm Poretic membrane before being used in the experiments.

Short-term uptake experiments—General experimental protocols: All the incubation and sampling experiments were performed in a class 100 laminar flow cabinet to avoid inadvertent trace metal contamination. The polycarbonate bottles for the diatom culture and uptake experiments were first soaked in acid and rinsed with Milli-Q water (18.0 MΩ) at least six times following trace metal clean techniques. The 0.22-μm filtered seawater used for the uptake media was first passed through a Chelex 100 ion exchange resin, with a removal efficiency higher than 95%, to remove any background Fe (data not shown). The ^{59}Fe radioisotope (in 0.1 mol L^{-1} HCl, New England Nuclear) was used to trace Fe uptake by the diatoms. Equimolar amounts of Fe (^{59}Fe in 0.1 mol L^{-1} HCl and stable Fe in 0.5 mol L^{-1} HCl) and Na_2-EDTA were mixed for 5 min. Afterwards, the mixture was spiked into the uptake medium with a nominal Fe concentration of 10 nmol L^{-1} (0.5 nmol L^{-1} ^{59}Fe and 9.5 nmol L^{-1} stable Fe) and with Na_2-EDTA. The HCl added with the mixture was neutralized with Suprapure NaOH (1 mol L^{-1}). These uptake media were equilibrated at the targeted temperature and irradiances overnight. The 1 : 1 ratio of Fe to Na_2-EDTA was used for a detectable ^{59}Fe count during the short-term uptake period. Although most of the Fe added should have precipitated under this concentration on the basis of the simple equilibrium calculations, the premix of Fe with EDTA repressed its precipitation because of the slow dissociation rate of Fe–EDTA complex (Hudson et al. 1992; Stumm and Morgan 1996; Kuma et al. 1999). Our preliminary experiments showed that most of the Fe added (>98%) was in the dissolved phase after equilibration. The radioactivity of ^{59}Fe was quantified at 1,072 keV with a Wallac gamma detector. The counting time was adjusted to result in propagated counting errors <5%.

The diatoms were first semicontinuously cultured under different environmental conditions (e.g., different temperatures or light intensities), depending on the different requirements of the experiment, in f/2 medium, except that the trace metal concentrations used were at the f/10 levels. The cell density was counted with a Coulter Counter every 12 h. After growing for several generations, the cells were collected, rinsed with the Fe-free 0.22-μm seawater, and then resuspended into the uptake medium. The short-term uptake experiments lasted for 1, 2, or 8 h, during which period samples were taken at several time points (e.g., four time points for short-term uptake under different irradiances). At each time point, a 10–20-mL (depending on exposure time and cell density) sample was filtered through the 1-μm polycarbonate membrane. The cell surface–adsorbed Fe was removed with the Ti solution (Hudson and Morel 1989). A 1-mL aliquot was also collected for measurement of total radioactivity in the uptake medium. The intracellularly accumulated Fe content was calculated as in Eq. 2.

$$\begin{aligned}\text{accumulated Fe content (nmol cell}^{-1}\text{)} &= [\text{radioactivity (cell}^{-1}\text{)} \\ &\quad \times \text{total metal concentration (nmol L}^{-1}\text{)}] \\ &\quad \div [\text{radioactivity of medium (L}^{-1}\text{)}] \end{aligned} \quad (2)$$

The Fe uptake rate was then obtained as the slope of the regression line between the intracellularly accumulated Fe and the exposure time, with an assumption that the potential cell growth and Fe efflux had a negligible effect on the linear relationship during the short exposure period. The *y*-intercept of the linear regression represents the transporter-bound Fe (Mirimanoff and Wilkinson 2000). Each uptake treatment had two replicates because the standard deviation for the algal uptake was small. With the assumption that the algae were under steady-state conditions, the intracellular Fe concentration could then be calculated by Eq. 1 with the efflux

rate constant neglected. Later, the intracellular Fe concentration with and without the consideration of efflux will be compared after the efflux rate constant is determined.

The algal cell sizes under different temperatures and irradiances were also quantified with a Coulter Counter. The cell sizes (diameters) were about 5 μm, with no significant difference in size among different treatments.

Uptake with refreshed medium: The diatom cells were first cultured at 19°C and 170 μmol photons m^{-2} s^{-1} with a 14 : 10 LD cycle. After they arrived at the midexponential phase, an 8-h short-term uptake experiment with 13 time points (0.25, 0.5, 0.75, 1, 2, 3, 3.25, 3.5, 3.75, 4, 5, 6, and 8 h) was performed. During this 8-h experimental period, the uptake medium was refreshed after 3 h because >70% (data not shown) of Fe was scavenged by the cells after 8 h without medium refreshing.

Uptake at different irradiances or temperatures: The algal cells were first acclimated at different irradiances or temperatures (40, 85, 170, and 340 μmol photons m^{-2} s^{-1} or 15°C, 19°C, and 24°C) for at least eight generations. Different irradiances were obtained by adjusting the distance from the light sources to the diatom culture. A 1-h short-term uptake experiment with several time points (0.25, 0.5, 0.75, 1 h for the irradiance experiment and 0.125, 0.25, 0.5, 0.75, 1 h for the temperature experiment) was then conducted after the diatoms reached the midexponential phase under different light intensities or temperatures. The intracellularly accumulated Fe and Fe uptake rate were then calculated.

Diel variation of Fe uptake: The cells were first acclimated at 19°C and 170 μmol photons m^{-2} s^{-1} at 14 : 10 LD for at least eight generations. After they arrived at the midexponential phase, a total of ten 1-h short-term Fe uptake experiments (five during the light period and five during the dark period) were performed continuously over about a 30-h period (i.e., from 11:00 h on the first day to 15:30 h on the second day) with 2- to 3-h intervals between each experiment. The cells for all 10 short-term uptake measurements were taken from the same bottle. When the uptake was performed during the dark period, all the bottles were covered with aluminum foil to minimize the potential effects of light.

Long-term uptake experiments—The long-term uptake experiments were performed in the same culture and uptake medium as those used during the acclimation period of the short-term uptake experiments. Different temperature or light intensity treatments were also the same as in the short-term uptake experiments. ^{59}Fe (0.92 nmol L^{-1}) was used to trace Fe accumulation, along with 2.33 μmol L^{-1} stable Fe (f/10 level). EDTA (100 μmol L^{-1}) was added to keep the free metal ion concentration constant during the experimental period. The pH of the medium was adjusted to 8.2 ± 0.1 with Suprapure NaOH (1 mol L^{-1}). The cell density was counted every day. At the same time, a 10–20-mL sample was filtered through the 1.0-μm polycarbonate membrane, soaked in Ti solution for 1 min (Hudson and Morel 1989), and rinsed with the 0.22-μm filtered seawater three times, and then the radioactivity was counted with the Wallac gamma detector. Another 5-mL sample without Ti wash was also collected to quantify the total cellular Fe accumulation. A 1-mL water sample was collected simultaneously for the measurement of total Fe radioactivity in the medium. The intracellular and total cellular Fe contents at each time point were calculated by Eq. 2. The difference between these two parameters indicated Fe that was loosely adsorbed to the cell surface. The Fe uptake rate was then calculated without considering the efflux rate constant first, similar to the short-term uptake experiment. The whole experiment lasted until the cells had acclimated for at least eight generations. During this period, the algal cells were subcultured once to avoid nutrient depletion. In the irradiance experiments, the subculture was performed on the third and fourth days for the two lower and higher irradiance treatments, respectively. In the temperature experiment, the subculture was conducted on the fifth day for all treatments.

Fe efflux from diatoms—The Fe efflux experiment was performed with ^{59}Fe-labeled diatoms resulting from the long-term Fe uptake experiments described previously. The Fe efflux for both living and dead cells was quantified. The algal cells were first collected, rinsed with Chelex resin–passed 0.22-μm filtered seawater three times, and then resuspended into the efflux medium. The dead cells were obtained after the diatoms were heated in a 50°C water bath for 10 min (Chen et al. 2003) and were kept intact as observed under the microscope. The efflux medium contained the Chelex resin–passed 0.22-μm filtered seawater with the addition of 5 μmol L^{-1} desferrioxamine B (DFB). The medium was equilibrated in the dark for a few hours before the start of the Fe efflux experiment. DFB is a trihydroxamate siderophore that specifically complexes inorganic Fe(III) with an extremely high conditional stability constant ($K^{\text{cond}}_{\text{FeL,Fe}} = 10^{16.5}$ L mol^{-1}) (Wells 1999 and references therein). A preliminary study conducted in our laboratory also demonstrated that Fe uptake by *T. pseudonana* was inhibited with the addition of DFB (Chen and Wang unpubl. data). Thus, DFB can prevent or at least minimize the reassimilation of eliminated Fe.

The efflux experiment lasted for 8 h with several time points of sampling. At each time point, 10- or 20-mL aliquots were removed, soaked in Ti-solution for 1 min, and rinsed with 0.22-μm seawater three times. The radioactivity of the cells was then counted, and the percentage of Fe retained in the cells was calculated. The total cellular Fe retained was also quantified with the 0.22-μm filtered seawater rinse only. The Fe retained in both the intracellular and total cellular pools were modeled with Eq. 3.

$$C_t = C_0 \times e^{-K_e t} \tag{3}$$

C_0 and C_t represent the cellular or intracellular Fe concentration at the beginning and at time t, respectively; k_e is the efflux rate constant; and t is the time of incubation/depuration.

Results

Short-term Fe uptake—Our preliminary experiment employed an 8-h exposure period to quantify Fe uptake. A hy-

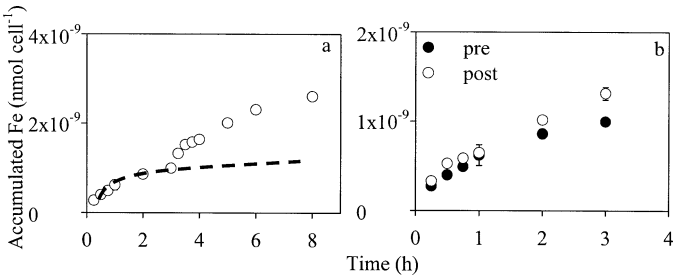

Fig. 1. (a) Increase in cellularly accumulated Fe concentration with exposure time during an 8-h period. Medium was refreshed after 3 h, and the dashed line represents Fe accumulation without medium refreshing. (b) Increase of cellularly accumulated Fe concentration during a 3-h period before and after medium refreshing. The cellularly accumulated Fe concentration before medium refreshing was subtracted from that after medium refreshing. Data are means ± semirange ($n = 2$).

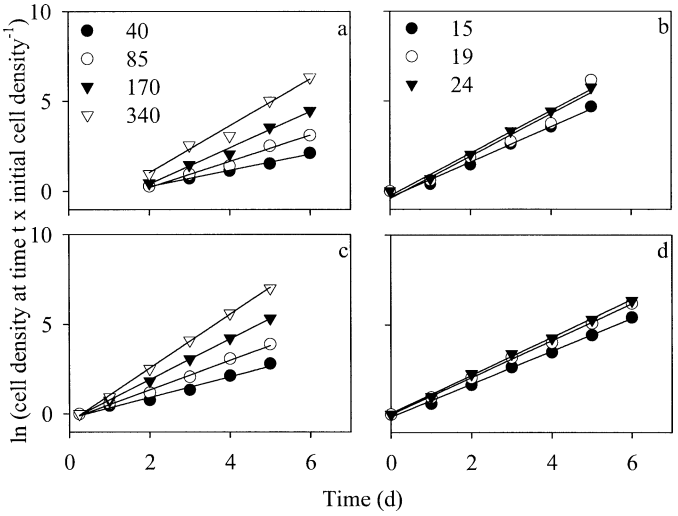

Fig. 2. Increase of ln (cell density at time $t \times$ [initial cell density]$^{-1}$) with the increase in growth time under (a, c) different irradiances (μmol photons m^{-2} s^{-1}) and (b, d) temperatures (°C) for both (a, b) the short-term and (c, d) long-term uptake experiments. The growth rate was quantified during the acclimation period for the short-term uptake experiments.

perbolic increase in intracellularly accumulated Fe with the increase of exposure time was observed, as shown by the dashed line in Fig. 1a. More than 70% of Fe in the uptake medium was scavenged by the algal cells at the end of 8 h. Thus, the Fe uptake experiment was then performed by renewing the medium after 3 h of exposure to examine whether the observed hyperbolic pattern was due to the marked decline of the ambient Fe concentration. In this experiment, the intracellularly accumulated Fe concentration first increased linearly and then started to level off after 3 h. When the medium was refreshed, there was a sudden increase and then a linear increase until the end of the 8-h uptake period. The intracellularly accumulated Fe increased by about 10 times (i.e., from 2.75×10^{-10} to 2.60×10^{-9} nmol cell^{-1}) from 0.25 h to 8 h of exposure. A good linear regression was obtained when data at the 3- and 8-h time points were excluded. A similar slope of the linear increase before and after medium renewal is shown in Fig. 1b. The intracellular Fe accumulation before medium renewal was subtracted from that after renewal. Thus, the intracellular Fe accumulation at each time point before and after medium renewal was comparable, suggesting that the decrease in ambient Fe concentration was responsible for the observed hyperbolic increase in intracellularly accumulated Fe concentration over the 8 h of exposure. The calculated transporter-bound Fe (i.e., the *y*-intercept of the linear regression between cellularly accumulated Fe and exposure time) before and after medium renewal was also comparable. In all subsequent experiments, uptake was performed within 1–2 h of exposure, and ambient Fe concentration during this relatively short period decreased <20%.

In the short-term uptake experiments, diatoms were first acclimated under different irradiances and temperatures for at least eight generations before the uptake experiments. There was a very quick response in the cell-specific growth rate (quantified during the acclimation period), which increased with an increase in light intensity or temperature (e.g., 0.51–1.3 d^{-1} when the light intensity increased from 40 to 340 μmol photons m^{-2} s^{-1}; Fig. 2a,b). The effect of temperature on cell growth was much less obvious than was the effect of irradiance, with a cell-specific growth rate of 0.98, 0.98, and 1.0 d^{-1} at 15°C, 19°C, and 24°C, respectively. The Fe accumulations quantified at different temperatures and light intensities are shown in Fig. 3. As expected, the intracellularly accumulated Fe concentration increased linearly with an increase in exposure time during the 1-h period (Fig. 3a,b), which further validated our assumption that the effects of cell growth and Fe efflux on cellular Fe accumulation could be neglected during a short period. For example, intracellularly accumulated Fe concentration increased from 5.53×10^{-10} to 1.05×10^{-9} nmol cell^{-1} at the lowest irradiance level. Similarly, intracellular Fe concentration increased linearly from 4.1×10^{-10} to 1.29×10^{-9} nmol cell^{-1} at the lowest temperature. At the same time, cellularly accumulated Fe concentration increased with an increase in light intensity at each of the four time points. For example, the cellularly accumulated Fe concentration increased from 1.05×10^{-9} to 1.89×10^{-9} nmol cell^{-1} after 1 h as the light intensity increased from 40 to 340 μmol photons m^{-2} s^{-1}. Intracellularly accumulated Fe concentration also increased slightly at the highest temperature treatment. Such a positive relationship was further evidenced by the calculated transporter-bound Fe and the Fe uptake rate, both of which increased with an increase in irradiance and temperature (Fig. 3c–f). For example, the transporter-bound Fe and Fe uptake rate were 1.83× and 1.78× higher for the highest compared with the lowest irradiance treatment. Because the cell-specific growth rate was higher under higher irradiance, intracellular Fe decreased 1.42× as the irradiance increased from 40 to 340 μmol photons m^{-2} s^{-1} (Fig. 3g). An opposite trend was found for the different temperature treatments as a result of the similar cell-specific growth rates under different temperatures. Accordingly, the intracellular Fe concentration increased slightly (1.18×) with an increase of temperature (Fig. 3h).

In the diel variation experiment, the diatom cells were

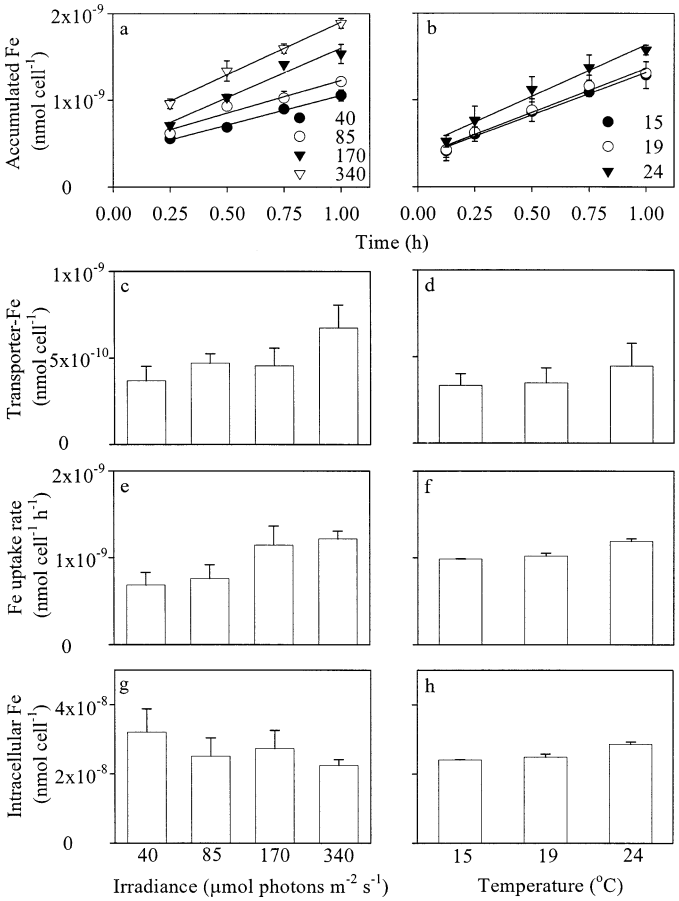

Fig. 3. (a, b) Increase of cellularly accumulated Fe concentration with exposure time under different irradiances (μmol photons m^{-2} s^{-1}) and temperatures (°C), respectively. (c, d) The transporter-bound and (g, h) intracellular Fe concentrations and (e, f) Fe uptake rates for cells under different irradiances and temperatures. Data are means ± semirange (n = 2).

maintained at a 14 : 10 LD cycle, and Fe accumulation was quantified at different periods of the day. The intracellularly accumulated Fe concentration proceeded linearly with exposure time for diatoms harvested at different diel periods (data not shown). The calculated Fe uptake rates (over 30 h) are shown in Fig. 4. Fe uptake rates ranged from 1.16 × 10^{-9} to 1.58 × 10^{-9} nmol cell^{-1} h^{-1} and were consistent with the results under different temperatures and light intensities (Fig. 3). Statistical analysis showed no diel variation in Fe uptake at different diel periods ($p > 0.05$, one-way ANOVA).

Long-term Fe uptake—The cell-specific growth rates of *T. pseudonana* under different temperatures and irradiances in the long-term experiment are shown in Fig. 2c,d. The growth rates were comparable to those observed in the short-term uptake experiments under the same conditions. For example, cell-specific growth rate increased from 0.63 (0.51) to 1.5 (1.30) d^{-1} when the ambient light intensity increased from 40 to 340 μmol photons m^{-2} s^{-1} in the long- (short-) term Fe uptake experiments. However, temperature had a much smaller effect on the cell-specific growth rate. It increased from 0.92 to 1.06 d^{-1} when the temperature increased from 15°C to 24°C.

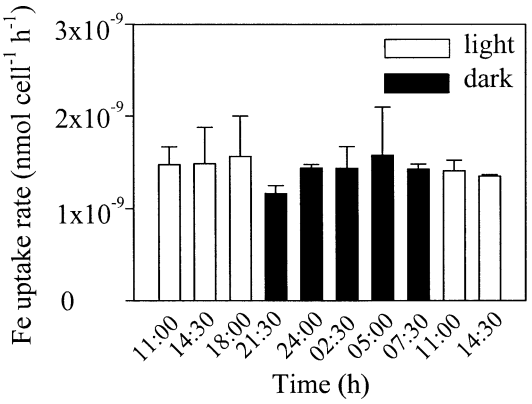

Fig. 4. Fe uptake rate at different times during a 30-h period with a 14 : 10 LD cycle. Data are means ± semirange (n = 2).

Because 100 μmol L^{-1} of EDTA was added to the long-term uptake medium, most of the Fe (77–81%) was present in the ambient water throughout the experiment, whereas 13–16% of Fe was adsorbed onto the cell surface and 6–7% was taken up into the cell after 5 and 6 d (Fig. 5a,b). The cell surface Fe adsorption, which accounted for 13–16% of the total Fe added, was completed within a few minutes (Hassler et al. 2004). Thus, bioavailable Fe concentrations were considered to be relatively constant during this exper-

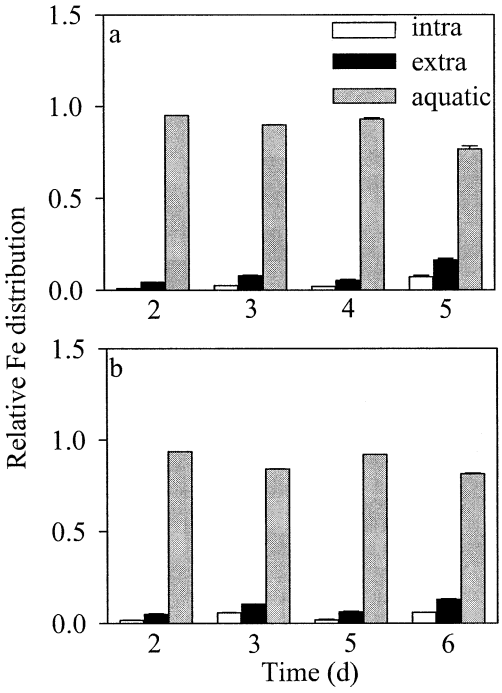

Fig. 5. Relative distribution of Fe in the cells (intra), on the cell surface (extra), and in the aquatic system (aquatic) for the long-term uptake experiment with (a) 170 μmol photons m^{-2} s^{-1} and (b) 19°C treatments as examples for the different irradiances and temperatures effects experiments. Data are means ± semirange (n = 2).

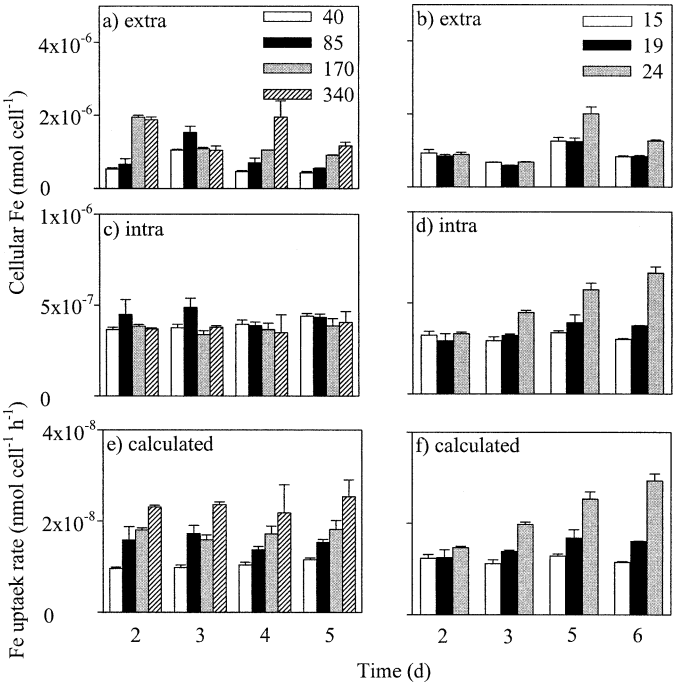

Fig. 6. (a, b) Cell surface–adsorbed (extra) and (c, d) intracellular (intra) Fe concentration and (e, f) the calculated Fe uptake rate at different time points for the different irradiance (μmol photons m^{-2} s^{-1}; panels a, c, e) and temperature (°C; panels b, d, f) effects experiments, respectively, in the long-term uptake experiment. Data are means ± semirange (n = 2).

iment, and steady-state condition was reached after several generations of growth.

Calculated Fe accumulation under different conditions is shown in Fig. 6. The cell surface–adsorbed Fe concentration increased when the cells were first inoculated into a new ^{59}Fe-labeled medium and then decreased continuously until the next subculture or medium renewal. Following a sudden increase after renewal, cell surface–adsorbed Fe decreased again, similar to the pattern observed before the subculture (Fig. 6a,b). For example, cell surface–adsorbed Fe concentration at the highest irradiance decreased from 1.88×10^{-6} to 1.04×10^{-6} nmol cell^{-1} before the subculture. Afterwards, it increased to 1.95×10^{-6} and decreased finally to 1.17×10^{-6} nmol cell^{-1}. Similar results were also observed in the other irradiance treatments and temperature experiments. However, this trend with time was not observed for intracellular Fe concentration and the Fe uptake rate. For most of the treatments in the irradiance and temperature experiments, both parameters remained almost constant and did not change with time (Fig. 6c–f). The only exception was the highest temperature treatment, in which intracellular Fe concentration increased from 3.31×10^{-7} to 6.64×10^{-7} nmol cell^{-1} and the Fe uptake rate increased from 1.47×10^{-8} to 2.93×10^{-8} nmol cell^{-1} h^{-1} as the exposure time increased from 2 to 6 d (Fig. 6d,f).

The final concentration of Fe loosely adsorbed to the cell surface, intracellular Fe concentration, and Fe uptake rates are compared for different irradiances and temperature treatments. Cell surface–adsorbed Fe concentration was higher

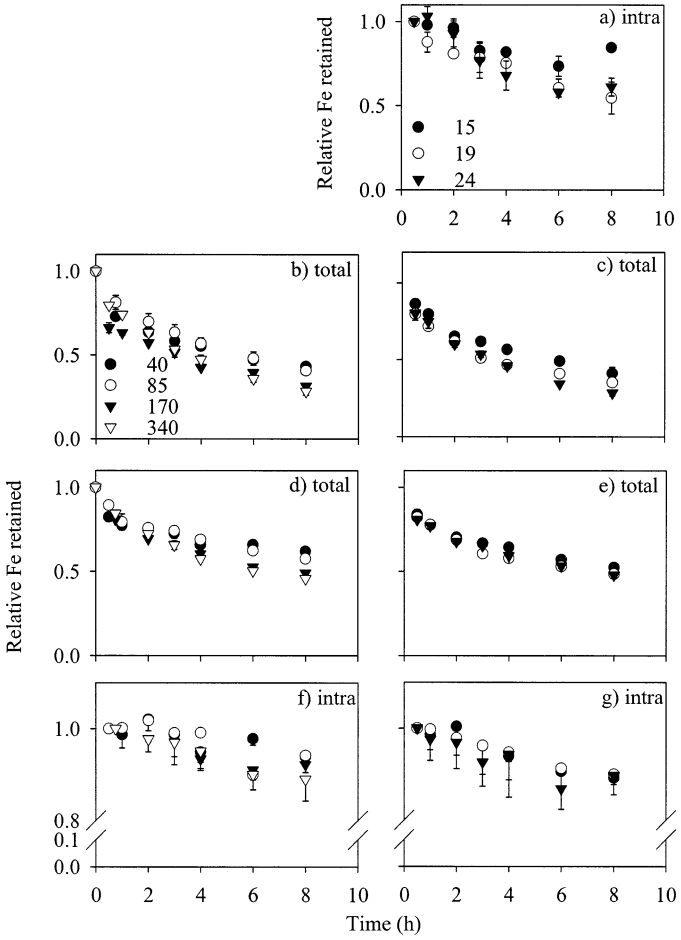

Fig. 7. Relative retention of intracellular (intra) and total cellular (total) Fe in (a–c) heat-killed and (d–g) living cells for different irradiances (μmol photons m^{-2} s^{-1}; panels b, d, f) and temperatures (°C; panels a, c, e, and g) during the 8-h depuration period. Data are means ± semirange (n = 2).

under higher irradiance (e.g., 4.28×10^{-7} and 1.17×10^{-6} nmol cell^{-1} under 40 and 340 μmol photons m^{-2} s^{-1}, respectively) or temperature (e.g., 8.29×10^{-7} and 1.26×10^{-6} nmol cell^{-1} at 15°C and 24°C, respectively; Fig. 6a,b). Intracellular Fe concentration remained constant at all four irradiance levels but was much higher at 24°C compared with the other two lower temperature treatments (Fig. 6c,d). Because of the higher cell-specific growth rate at a higher light intensity, the calculated Fe uptake rate increased with an increase in irradiance (Fig. 6e). The Fe uptake rate increased by 2.2× as irradiance increased from 40 to 340 μmol photons m^{-2} s^{-1}. Similarly, the Fe uptake rate increased with an increase in temperature (Fig. 6f). When temperature increased from 15°C to 24°C, the calculated Fe uptake rate increased from 1.15×10^{-8} to 2.93×10^{-8} nmol cell^{-1} h^{-1}.

Fe efflux from the diatoms—Figure 7 shows the relative Fe concentration decreases in both living and heat-killed cells under different irradiances and temperatures. The dead cells showed a remarkable decrease in both total (by 57–

Table 1. Fe efflux rate constant (k_e) for total and intracellular (intra) pools of heat-killed and living *Thalassiosira pseudonana* diatoms. Mean ± semirange ($n = 2$).

		Cells	k_e (h^{-1})	r^2	p
Irradiance	40	Dead (total)	0.092±0.016	0.875	<0.01
(μmol photons m^{-2} s^{-1})	85		0.106±0.009	0.968	<0.001
	170		0.120±0.018	0.883	<0.001
	340		0.149±0.009	0.980	<0.001
	40	Living (total)	0.048±0.011	0.770	<0.01
	85		0.061±0.008	0.910	<0.001
	170		0.082±0.011	0.912	<0.001
	340		0.095±0.010	0.948	<0.001
	40	Living (intra)	0.008±0.003	0.567	0.051
	85		0.013±0.004	0.629	<0.05
	170		0.013±0.003	0.823	<0.05
	340		0.017±0.002	0.964	<0.001
Temperature (°C)	15	Dead (total)	0.103±0.010	0.947	<0.001
	19		0.121±0.015	0.916	<0.001
	24		0.151±0.010	0.974	<0.001
	15	Dead (intra)	0.059±0.007	0.943	0.001
	19		0.075±0.006	0.966	<0.001
	24		0.081±0.014	0.867	<0.010
	15	Living (total)	0.070±0.010	0.891	<0.001
	19		0.081±0.012	0.881	<0.001
	24		0.081±0.011	0.905	<0.001
	15	Living (intra)	0.017±0.002	0.928	<0.001
	19		0.015±0.001	0.986	<0.001
	24		0.016±0.004	0.792	<0.010

72%) and intracellular Fe concentrations (by 40%) during the 8-h efflux period (Fig. 7a–c). Similarly, total cellular Fe concentration decreased notably during the experimental period with living cells. The relative Fe retained at the end of 8 h ranged from 45.7% to 62% and 48% to 52.5% under different irradiances and temperatures, respectively (Fig. 7d,e). Fe elimination from intracellular pools of living cells was smaller compared with dead cells. The relative decrease ranged from 5.8% to 11% and 10% to 11% for different irradiances and temperatures, respectively (Fig. 7f,g).

The Fe elimination of the two different pools (i.e., intracellular and total cellular) followed an exponential pattern for both dead and living cells, as described by Eq. 3. The calculated efflux rate constants (k_e) under different light and temperature conditions are shown in Table 1. Fe elimination of both pools was faster for dead cells compared with living cells under the same irradiance and temperature. Calculated k_e for total cellular Fe of dead cells was about 1.6× higher than that of living cells under the highest irradiance of 340 μmol photons m^{-2} s^{-1} (i.e., 0.149 compared with 0.095 h^{-1}). Furthermore, the k_e for intracellular Fe of dead cells was about 5× higher than that for living cells at 24°C (i.e., 0.081 compared with 0.016 h^{-1}). On the other hand, Fe elimination from both pools of dead and living cells was higher under the higher irradiance and temperature conditions, except for the intracellular pools of living cells under different temperatures. For example, in a temperature experiment with dead cells, 41.2% total cellular Fe was retained after 8 h at 15°C, whereas only 28.4% was retained at 24°C (Fig. 7c). Such a trend of Fe elimination was also demonstrated by the increase of Fe k_e with an increase in temperature and irradiance (Table 1). However, intracellular Fe elimination of living cells was relatively constant under different temperatures, with the k_e values ranging from 0.015 to 0.017 h^{-1}.

Discussion

Fe accumulation under different temperatures and irradiances—Previous studies employed either long-term or short-term experiments to quantify Fe uptake rates by phytoplankton, but comparison of these two methods in calculating Fe uptake has not yet been directly conducted. Our quantified Fe accumulations in short-term and long-term uptake experiments were generally comparable to the results of the previous studies employing similar bioavailable Fe concentrations (e.g., Sunda and Huntsman 1995; Chen and Wang 2001). In our study, the trends obtained from both methods were mostly consistent. Because different total Fe and EDTA concentrations were used in the short-term and long-term uptake experiments (i.e., 10 nmol Fe L^{-1} vs. 2.33 μmol Fe L^{-1}; 10 nmol EDTA L^{-1} vs. 100 μmol EDTA L^{-1}), the bioavailable Fe concentrations were completely different. Consequently, the absolute values of Fe uptake rate, cellular Fe concentration, and other parameters varied by orders of magnitude between these two experiments.

Light and temperature are both important environmental variables and play important roles in Fe–phytoplankton interactions by affecting the ambient bioavailable Fe concentration and the physiology of phytoplankton. Light can induce Fe photoreduction, and temperature can affect the thermal formation/dissociation of organic Fe complex as well as the oxidation of photoproduced Fe(II) (Sunda and Huntsman 2003). Both parameters can also affect the bio-

chemical composition and cell volume of phytoplankton. For example, cellular N, chlorophyll *a*, and C : N ratio increase with increasing temperature (Berges et al. 2002). Meanwhile, phytoplankton acclimated at lower light intensity tend to have a higher Fe requirement in the photosynthetic system, similar to cells acclimated at higher temperature. Furthermore, the effects of temperature and irradiance on Fe accumulation and cell surface adsorption might be dependent on cellular Fe quota. Therefore, Fe accumulation in phytoplankton at different light levels and temperatures is complicated, and contrasting conclusions have been drawn in previous studies.

Both short-term and long-term uptake experiments showed that Fe uptake rate was higher under higher irradiance, whereas intracellular Fe concentration was slightly decreased (short-term experiment) or relatively constant (long-term experiment) with an increase in light intensity. The effects of irradiance on Fe uptake and cellular Fe concentration have been examined in a few previous laboratory and field studies (Sunda and Huntsman 1997; Schmidt and Hutchins 1999; Strzepek and Price 2000), with no generally consistent result. Both parameters either increased, remained constant, or even decreased with an increase in irradiance. Raven (1990) calculated that more Fe was required at a lower photon flux density (i.e., irradiance) for a much higher pigment content to intercept more light energy. Our results suggested that the intracellular Fe concentrations under the four different irradiances were not as different as we had thought before.

Several mechanisms could explain our results. First, diatoms might supply enough Fe for extra pigment synthesis under lower irradiance by transporting Fe from other organelles to the thylakoid, where the photosynthetic system is located. For example, 50% of cellular Fe occurred within photosynthetic units at high light, whereas 90% occurred at low light (Sunda and Huntsman 1997). Second, the coastal diatom *T. pseudonana* can take up luxury Fe. Cellular Fe concentration can be 20–30 times higher than required for maximum growth (Sunda and Huntsman 1995). Thus, abundant Fe storage in the cells was sufficient for pigment synthesis even under low light intensity. Third, the light intensity used in our study might be not low enough to result in an increase in intracellular Fe concentration. A threshold irradiance above which phytoplankton can complete extra pigment synthesis is possible with Fe redistribution or utilization of Fe storage. Below this threshold level, the diatoms might have to increase their Fe uptake rate or further decrease their cell-specific growth rate to obtain a high intracellular Fe concentration for normal physiological function. Strzepek and Price (2000) found that a very high intracellular Fe concentration occurred only at an extremely low light intensity. Finally, more Fe might be bioavailable at the higher light intensity because of photoreduction (Price and Morel 1998; Barbeau et al. 2001), which could further complicate the results.

Recently, Rijkenberg et al. (2005) reported a diel cycle of bioavailable Fe concentrations, with the highest Fe concentration at noon as a result of photoreduction, which subsequently induced Fe uptake in the daytime. Concurrently, the Fe requirement was higher during the daytime as cellular chlorophyll levels reached their minimum around the middle of the dark period and began to increase before the photoperiod (Falkowski and La Roche 1991). However, diel Fe uptake was rather constant during the 30-h period in our study. Consequently, the biological demand for Fe by the diatoms is probably decoupled from Fe uptake.

Temperature effects have been much less examined. In both short-term and long-term Fe uptake experiments, the Fe uptake rate as well as the intracellular Fe concentration was higher at 24°C, although such a trend was less obvious in the short-term experiment. Strzepek and Price (2000) found that the Fe uptake rate, as well as the cell-specific growth rate, for *Thalassiosira weissflogii* increased proportionally with an increase in temperature, resulting in a similar intracellular Fe concentration under different temperatures. However, phytoplankton growth at high temperature mimics low light acclimation (Maxwell et al. 1994), and there are more photosynthesis II reaction centers, leading to a greater Fe requirement (Davison 1991). This could explain the higher intracellular Fe concentration and Fe uptake rate at higher temperatures observed in our study. Furthermore, Sunda and Huntsman (2003) found that more Fe was bioavailable under the lower temperature conditions because of its different effects on photochemical and thermal reaction rates. Thus, the increase in intracellular Fe concentration and uptake with the increase in temperature might have even been underestimated in our study.

Fe efflux under different temperatures and irradiances—A remarkable Fe efflux from intracellular pools was observed in our study, with the efflux rate constant k_e ranging from 0.008 to 0.017 h^{-1}. In our study, any Fe excreted was trapped by DFB (Wells 1999) to minimize the reassimilation of Fe by the diatoms. The k_e observed in our study could be underestimated if there was a significant Fe redistribution/internalization from the cell surface to the intracellular compartments during the depuration period (Hudson and Morel 1990). The cell surface–adsorbed Fe was not removed before the start of the efflux experiment because the Ti reagent might have damaged the living cells and further affected Fe elimination (Sunda and Huntsman 1995). Fe release from the whole cells was also considerable, with a k_e estimate of 0.048–0.095 h^{-1} for the living cells. Theoretically, Fe release from the whole cell included both Fe efflux from the intracellular compartments and Fe dissociation from the cell surface. Because most accumulated Fe was distributed on cell surfaces, the Fe efflux from intracellular pools can be neglected and k_e for Fe elimination from the whole cell can be considered as the Fe dissociation from the cell membrane. This hypothesis was supported by the increase of k_e with the increase in irradiance or temperature because the dissociation is energy dependent (Kuma et al. 2000; Sunda and Huntsman 2003).

Measurements of the loss of Fe from dead cells were designed to serve as a control for monitoring the release of Fe from the cells only as a result of Fe dissociation. Gross Fe elimination from the intracellular compartments could be calculated as the difference between loss from the dead cells (i.e., Fe dissociation from cell surfaces only) and Fe efflux from the whole living cell. However, Fe elimination from

Table 2. Comparison of intracellular Fe concentration and Fe uptake rate with and without considering Fe efflux.

Parameter	Environmental variable		Without efflux	With efflux
Intracellular Fe conc. in short-term uptake (nmol cell^{-1})	Irradiance (μmol photons m^{-2} s^{-1})	40	3.23×10^{-8}	2.36×10^{-8}
		85	2.53×10^{-8}	1.76×10^{-8}
		170	2.75×10^{-8}	2.09×10^{-8}
		340	2.24×10^{-8}	1.71×10^{-8}
	Temperature (°C)	15	2.41×10^{-8}	1.70×10^{-8}
		19	2.50×10^{-8}	1.83×10^{-8}
		24	2.86×10^{-8}	2.06×10^{-8}
Fe uptake rate in long-term uptake (nmol cell^{-1} h^{-1})	Irradiance (μmol photons m^{-2} s^{-1})	40	1.15×10^{-8}	1.50×10^{-8}
		85	1.53×10^{-8}	2.09×10^{-8}
		170	1.82×10^{-8}	2.32×10^{-8}
		340	2.53×10^{-8}	3.22×10^{-8}
	Temperature (°C)	15	1.15×10^{-8}	1.66×10^{-8}
		19	1.60×10^{-8}	2.16×10^{-8}
		24	2.93×10^{-8}	3.99×10^{-8}

heat-killed cells was even faster than that from living cells, indicating the characteristic change of cell surface metal-binding ligands after heating (i.e., 0.092–0.151 h^{-1} compared with 0.048–0.095 h^{-1}). Moreover, there was also a notable decrease in intracellular Fe concentration in heat-killed cells, with k_e ranging from 0.059 to 0.081 h^{-1}. Thus the membrane permeability of diatoms might have changed, although the heat-killed cells were kept intact (observed under the microscope). In addition, Fe dissociation from the cell surface might be overestimated because of DFB competition binding (Borer et al. 2005). However, Fe release from the intracellular compartments of living cells was not affected because neither the DFB–Fe complex (Wells 1999; Kuma et al. 2000) nor DFB was directly bioavailable, at least for this algal species.

Potential growth dilution of the intracellular Fe concentration was subtracted from the results, given the significant cell growth during the efflux experiment ($\sim$1.3$\times$ increase in cell density). Without calibration for growth dilution, the decrease in the intracellular Fe concentration during the 8-h period would be more obvious. Thus, the relative Fe decrease from intracellular compartments can be considered as the true Fe efflux out of the cells, which might even be underestimated in our study. The efflux rate constant k_e was often neglected compared with the cell-specific growth rate (μ) when calculating cellular metal concentration for the phytoplankton (Miao and Wang 2004). Our results implied that k_e was significant (i.e., k_e was 0.008–0.017 h^{-1} compared with a μ of 0.026–0.063 h^{-1}) and cannot be neglected, at least for the case of Fe. The intracellular Fe concentrations or Fe uptake rates before and after consideration of the efflux rate constant k_e were compared in Table 2 for the short- or long-term experiment. A notable difference was found with and without k_e, although the trends for different temperatures and irradiances were similar.

Our study demonstrated a significant Fe efflux in marine diatoms. Because no Fe efflux data were available for marine phytoplankton from the literature, our results were compared with the Fe efflux from macrophage, cardiac myocytes, and Xenopus oocytes (Donovan et al. 2000; Liu et al. 2003; Chung et al. 2004). Because different timescales were used and only the relative percentage of Fe decrease was available in these previous studies, the k_e was calculated with the assumption that Fe release from the cell follows first-order kinetics. The k_e values ranged from 0.013 to 0.038 h^{-1} for macrophages (Rama et al. 1988; Chung et al. 2004), from 0.006 to 0.009 h^{-1} for cardiac myocytes (Liu et al. 2003), and from 0.001 to 0.013 h^{-1} for Xenopus oocytes (Donovan et al. 2000). Thus, Fe efflux measured in our study was in agreement with these different cell types. Fe dissociation from the cell surface in this study was also compared with other studies. The rate constant for Fe dissociation from the Fe transporter on the cell surface was about 0.72 h^{-1} for the coccolithophorid *Pleurochrysis carterae* (Hudson and Morel 1990), which was much higher than observed in our study for the whole binding ligands of *T. pseudonana*. The rate constants of total Cd export from the marine diatom *T. weissflogii* and Zn export from *Chlorella kesslerii* were 0.11–0.16 h^{-1} and 0.084–0.222 h^{-1} for Cd and Zn, respectively (Lee et al. 1996; Hassler and Wilkinson 2003), which were somewhat higher than the Fe dissociation (i.e., 0.048–0.095 h^{-1}) obtained in our study. Thus, metal dissociation from the cell surface could be specific to algae, metal species, and binding ligands.

Two possibilities could explain the significant Fe efflux from the diatoms. On the one hand, the diatom used in our study is a coastal species, which was rarely limited by Fe, and thus highly efficient usage of intracellular Fe was probably not well developed. On the other hand, the high Fe efflux might also have some advantages with respect to other organisms. Because of a luxury Fe uptake in marine phytoplankton (Sunda and Huntsman 1995), the cells might first take up much more Fe than necessary for their biological requirements when the Fe concentration in the environment is high (e.g., episodic aeolian deposition). Subsequently, Fe might be excreted out of the cells in the form of more dissolved and bioavailable species, which can be taken up again by the phytoplankton, as well as other organisms (Hutchins et al. 1993). Thus, the phytoplankton can act as a buffer system for Fe. Gordon et al. (1997) calculated that only 4–20% of total primary productivity was supplied by the new iron and that each individual iron atom can be cycled in the surface water 169 times before being removed in sinking particles. Our results further prove the importance of Fe re-

generation in the surface water, but the forms of Fe that are excreted need to be further examined.

Although more Fe might be required under low irradiance for the synthesis of more pigments to intercept enough light energy, Fe uptake rate was much lower at low irradiance on the basis of the results of both short- and long-term uptake experiments. Under this condition, the diatoms had to decrease cell-specific growth rate μ to keep a relatively high intracellular Fe concentration. Consequently, the Fe uptake rate was decoupled with the different Fe requirements under different irradiances. This was further evidenced by the relatively constant Fe uptake rate in the diel variation experiment. Several other mechanisms (e.g., intracellular Fe redistribution and luxury uptake) might be involved to fulfill the higher Fe requirement under low irradiance. An opposite trend was found for temperature. The higher Fe requirement at high temperature was mainly fulfilled by an increased Fe uptake rate, whereas the cell-specific growth rate was kept relatively constant. Although the Fe efflux rate constant plays an important role in the calculation of intracellular Fe concentration and Fe regeneration in the ocean, it has a negligible effect for different Fe requirements under different temperatures and irradiances.

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