Limnol. Oceanogr., 50(4), 2005, 1181–1192

Effect of CO_2 supply and demand on zinc uptake and growth limitation in a coastal diatom

William G. Sunda[1] *and Susan A. Huntsman*
Center for Coastal Fisheries and Habitat Research, National Ocean Service, National Oceanic and Atmospheric Administration, Beaufort, North Carolina 28516

Abstract

We conducted culture experiments with *Thalassiosira pseudonana* to determine the effect of CO_2, photoperiod, and light intensity on cellular zinc concentrations and zinc requirements for growth. Cellular zinc requirements were dependent on the supply of CO_2 relative to its photosynthetic demand. Decreasing the CO_2 concentration (via an increase in pH from 8.2 to 9.0) increased the cellular zinc required to achieve a given growth rate or that needed for maximum growth. This increase is apparently linked to a greater demand for the zinc enzyme carbonic anhydrase (CA), which is needed for cellular CO_2 acquisition. A decrease in photoperiod had a similar effect. Based on the present and previous results, a decrease in photoperiod from 24 h d^{-1} (continuous light) to 7 h d^{-1} was accompanied by an estimated 2.2-fold increase in the net specific rate of photosynthetic C fixation, which increased the cellular demand for CA. The higher cellular requirement for zinc under decreased CO_2 or photoperiod was accentuated at high growth rates because of a disproportionate increase in the cellular demand for CA with increasing specific rate of C fixation. The increased demand for cellular zinc was largely met by a decrease in the daily specific growth rate, which increased cellular zinc concentrations by decreasing biodilution rates. In addition, there was an approximately twofold increase in cellular zinc uptake rates at high zinc concentrations (and high growth rates) for cells grown at low CO_2 concentration. In contrast to the effects of decreased CO_2 or photoperiod, a tenfold decrease in light intensity reduced the cellular zinc requirement, apparently linked to a 2.8-fold decrease in the maximum specific growth rate, and resultant decreased demand for CA and other biosynthetic zinc enzymes. Other factors (e.g., iron limitation) that decrease specific growth rate should have a similar effect.

Zinc is an essential nutrient for phytoplankton, but it can also inhibit growth at elevated concentrations (Anderson et al. 1978; Sunda and Huntsman 1992, 1995, 2000). There is evidence that zinc limits phytoplankton productivity in some regions of the ocean, such as the sub-Arctic Pacific (Coale 1991; Crawford et al. 2003) and coastal waters off California and Costa Rica (Franck et al. 2003). In addition, zinc limitation can affect the composition and structure of marine planktonic communities because of differences in zinc requirements among phytoplankton species (Brand et al. 1983; Sunda and Huntsman 1995). In the sub-Arctic Pacific, zinc addition preferentially stimulated the growth of coccolithophores relative to that of noncalcifying phytoplankton, suggesting that zinc may influence seawater alkalinity, and hence air–sea exchange of CO_2 (Crawford et al. 2003).

As with major nutrients, algal uptake of zinc and subsequent settling of biogenic particles exerts a dominant controlling influence on zinc concentrations and availability to phytoplankton. This is evidenced by the strong concomitant depletion of zinc and nutrients (N and P) in surface ocean waters (Bruland 1980) and in eutrophic estuaries during algal blooms (Luoma et al. 1998). Consequently, environmental variables that influence cellular zinc concentrations in phytoplankton will also affect zinc cycling and distributional patterns in both oceanic and coastal waters.

Zinc is an essential functional component of many cellular enzymes (da Silva and Williams 1991). One of most abundant zinc enzymes in phytoplankton is carbonic anhydrase (CA), which catalyzes the interconversion of bicarbonate ions and CO_2 (Morel et al. 1994). This enzyme is found in a variety of cellular compartments, including the periplasmic space next to the outer cell membrane, and within the chloroplast at the site of CO_2 fixation by Rubisco (Badger and Price 1994). Carbonic anhydrase is required for the uptake and fixation of inorganic carbon, especially at low CO_2 concentrations at the cell surface (Badger and Price 1994; Lane and Morel 2000; Burkhardt et al. 2001). Consequently, high concentrations of CA and cellular Zn are needed for algal growth at low CO_2 availability, and hence CO_2 and zinc may be colimiting in some oceanic environments (Morel et al. 1994).

A second important function of zinc is in proteins involved in DNA metabolism (da Silva and Williams 1991). These include DNA and RNA polymerases, reverse transcriptase, and zinc finger transcription factors. Because of zinc's essential role in DNA metabolism, zinc-limited *Eulgena* cells were incapable of completing their mitotic cycle and were unable to undergo cell division (Falchuk et al. 1975). Zinc may also be involved in silica uptake and frustule deposition in diatoms, as evidenced by decreased silica uptake rates and Si : P ratios at low dissolved inorganic zinc (Zn') concentrations (Rueter and Morel 1981; Ellwood and Hunter 2000).

Because of the presence of zinc in carbonic anhydrase and the critical role of this enzyme in CO_2 acquisition and fixation, the zinc requirement in phytoplankton should be affected by the supply of CO_2 relative to its demand. Decreases

[1] Corresponding author (bill.sunda@noaa.gov).

Acknowledgments

This work was partially funded by grants from the Office of Naval Research. We thank Lisa Lowry for technical assistance, John Hare for help with statistical analyses, and Wayne Litaker and two anonymous referees for helpful reviews of the manuscript.

in CO_2 concentration increase the growth requirement for CA and zinc, as demonstrated in the coastal diatom *Thalassiosira weissflogii* (Morel et al. 1994; Lane and Morel 2000). Variations in light intensity or photoperiod should also affect zinc requirements by influencing the rate of photosynthesis and thus the metabolic demand for CO_2. Decreasing the light intensity decreases photosynthetic C-fixation rates, which lowers CA activities in phytoplankton (Berman-Frank et al. 1995), and thus should decrease zinc requirements. Decreasing photoperiod should have the opposite effect since it increases the specific rate of carbon fixation in *Thalassiosira pseudonana* and other diatoms (Hobson et al. 1985; Sunda and Huntsman 2004). However, the picture is complicated by the fact that different interactive effects may occur with regard to the demand for zinc in other zinc proteins, such as those involved in DNA replication and transcription.

Here we present the results of experiments that examine the effect of CO_2 concentration, light intensity, and photoperiod on zinc limitation of growth rate and zinc uptake in the coastal diatom *T. pseudonana*. Relationships were determined among key independent and dependent variables: the concentration of dissolved inorganic zinc species (which controls zinc uptake by phytoplankton), cellular zinc uptake rate, cellular zinc concentration, carbonic anhydrase activity, chlorophyll *a* (Chl *a*), and specific growth rate.

Methods

Experiments were conducted with *T. pseudonana* in chemically defined culture media at 20°C. The experiments used methods similar to those in previous algal studies with Zn (Sunda and Huntsman 1992, 1995, 1998, 2004).

Four experiments were conducted. Experiments 1 and 2 examined the effect of pH and consequent changes in CO_2 concentration. In these experiments cells were grown in seawater media at ambient pH (8.2) and CO_2, and in media in which the CO_2 was decreased substantially by adjustment of pH to 9.0 via addition of NaOH (*see below*). These experiments were conducted at a saturating light intensity (500 μmol photons m^{-2} s^{-1}) and a photoperiod of 14 h d^{-1}, the light conditions used in most of our previous culture studies (Sunda and Huntsman 1992, 1995, 1998, 2000). Light was provided by Vita-Lite fluorescent bulbs (Duro Test).

The effect of light intensity and photoperiod were examined in two subsequent experiments (3 and 4) conducted at ambient seawater pH (8.2). In experiment 3, cells were grown under the same 14-h photoperiod as used in experiments 1 and 2, but at a tenfold lower light intensity (50 μmol quanta m^{-2} s^{-1}). In experiment 4, the cells were grown under continuous light at the same saturating light intensity (500 μmol quanta m^{-2} s^{-1}) as in experiments 1 and 2. The results of this experiment and those from experiment 1 (pH 8.2) were compared with previously reported results for *T. pseudonana* cultures grown at the same pH and light intensity but with a severely shortened (7 h) photoperiod (Sunda and Huntsman 2004).

Media preparation and composition—The cultures were grown in 500-ml polycarbonate bottles containing 320–490 ml of nutrient-enriched offshore seawater (salinity 36). The seawater was collected from the Gulf Stream with a peristaltic pumping system and stored in the dark before use. To prepare media, the seawater was passed through 0.4-μm pore Nuclepore filters and enriched with 32 μmol L^{-1} $NaNO_3$, 2 μmol L^{-1} Na_2HPO_4, 40 μmol L^{-1} Na_2SiO_3, 10 nmol L^{-1} Na_2SeO_4, and vitamins (0.074 nmol L^{-1} vitamin B_{12}, 0.4 nmol L^{-1} biotin, and 60 nmol L^{-1} thiamin).

Trace metal ion buffer systems were added to control and quantify concentrations of free trace metal ions and dissolved inorganic metal species. These systems consisted of 100 μmol L^{-1} ethylenediaminetetraacetic acid (EDTA) and 3–100 nmol L^{-1} Zn, 40 nmol L^{-1} Cu, 48 nmol L^{-1} Mn, and 1.0 μmol L^{-1} Fe. Cobalt, which can nutritionally substitute for zinc (Sunda and Huntsman 1995), was not added. The computed ratios of total metal to free metal ion concentration at pH 8.2 were $10^{3.99}$, $10^{1.23}$, and $10^{6.12}$ for Zn, Mn, and Cu, respectively (Sunda and Huntsman 1992, 1995). The value at pH 9.0 was 0.09 log units higher because of the influence of elevated pH on free ion concentrations of calcium and magnesium ions, which compete with the trace metals for complexation with EDTA (Price et al. 1988/89). At growth limiting concentrations, algal zinc uptake is controlled by the concentration of kinetically labile dissolved inorganic zinc species (Sunda and Huntsman 1992), which include the aquated free zinc ion (Zn^{2+}), and lesser concentrations of inorganic complexes with chloride ($ZnCl^+$), hydroxide ($ZnOH^+$), carbonate ($ZnCO_3$), and sulfate ($ZnSO_4$) (Byrne et al. 1988). The ratio of the concentration of dissolved inorganic zinc species (Zn′) to free aquated zinc ions (the inorganic side reaction coefficient, α_{Zn}) is 1.51 at pH 8.2 (Byrne et al. 1988). This ratio increases to 2.27 at pH 9.0 due to increased complex formation by hydroxide and carbonate ions. Algal zinc uptake and growth limitation are plotted as functions of the Zn′ concentration ([Zn′]), computed by multiplying [Zn^{2+}] by the computed values of α_{Zn}.

Total metal concentrations in the media were computed from the sum of the concentration of added metal salt, the estimated background concentration in the culture medium, and the measured concentrations of metal added with radiotracer stock solutions. Background concentrations of Zn, Cu, and Mn in the culture medium are estimated to be 1, 1, and 2 nmol L^{-1}, respectively, based on previous measurements in Gulf Stream seawater and the concentrations added with the stock solutions of EDTA and Chelex-treated nutrients (Sunda and Huntsman 1998).

Most culture media were labeled with ^{65}Zn to measure cellular zinc concentrations (Sunda and Huntsman 1992). In addition, the media in experiment 1 were also labeled with ^{109}Cd to simultaneously measure both cellular Zn and Cd (Sunda and Huntsman 1998). (The cellular Cd data from this experiment will be reported in a subsequent paper.) In this experiment 0.18 nmol L^{-1} Cd was added to the medium in conjunction with ^{109}Cd, yielding a computed [Cd′] of 1.0 pmol L^{-1} and a [Cd^{2+}] of 0.03 pmol L^{-1}. Although Cd can partially substitute for Zn in some marine algae (Lee and Morel 1995; Lee et al. 1995), we have found little evidence for such replacement in *T. pseudonana* (Sunda and Huntsman 1998). In addition, the measured cellular Cd : C quotas that we measured in experiment 1 at pH 8.2 (0.170, 0.093,

and 0.0042 μmol mol^{-1}, respectively, at [Zn′] of 0.9, 3.4, and 13 pmol L^{-1}; unpubl. data) should be too low to have any effect on cellular zinc requirements even if *T. pseudonana* were capable of Cd replacement.

In experiments 3 and 4, one set of culture media was labeled with ^{65}Zn, while a replicate set was labeled with ^{14}C-bicarbonate. The ^{14}C bottles were used to measure cellular carbon concentrations (*see below*).

Culture pH and CO_2—In the CO_2 effects experiments (1 and 2) the cells were grown in media at ambient pH (8.2) and CO_2 and in media in which the CO_2 was decreased ~11-fold by adjusting the pH to 9.0 via addition of Suprapur NaOH (Merck). Following the addition of NaOH, the media were allowed to equilibrate at 20°C overnight before cell inoculation. The high-pH cultures were grown in tightly capped, 500-ml polycarbonate bottles with <10% head space to minimize increases in CO_2 concentration via diffusion from the atmosphere. The ^{14}C-labeled culture bottles in experiments 3 and 4 were also tightly capped to minimize loss of ^{14}C. However, for ambient pH cultures labeled with ^{65}Zn, the caps were loosened to allow CO_2 exchange with the atmosphere. All experiments were conducted at low cell densities (*see below*) to prevent algal C fixation from substantially depleting CO_2 and increasing culture pH.

In experiments 1 and 2, culture pH was measured immediately after inoculation, at the time of cellular ^{65}Zn sampling, and 2–3 times in between. In the high-pH bottles, the mean pH was 9.01 ± 0.03 (±SD) in experiment 1 and 9.02 ± 0.03 in experiment 2. The mean pH in the ambient pH bottles was 8.20 ± 0.06 and 8.22 ± 0.04, respectively, in experiments 1 and 2. For the two light experiments (3 and 4) the mean pH was 8.20 ± 0.04 and 8.19 ± 0.05. For these experiments the pH was measured only at the time of cellular ^{65}Zn or ^{14}C measurement.

Based on the total inorganic carbon (TIC) concentration of the Sargasso Sea water and equilibrium calculations (Goyet and Poisson 1989), the CO_2 concentration was ~10 μmol L^{-1} at a pH of 8.2 and ~0.9 μmol L^{-1} at pH 9.0. The TIC concentration (2.2 mmol L^{-1}) is based on the value for seawater at a salinity of 36 and a pH of 8.2 (Stumm and Morgan 1981; Goyet and Poisson 1989). Culture pH was measured against National Bureau of Standards pH standards with an Accumet combination electrode (Sunda and Huntsman 2003).

Experimental procedures and analyses—The coastal diatom, *T. pseudonana* (clone CCMP 1335, formerly 3H), was obtained as an axenic culture from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton, West Boothbay Harbor, Maine. It was maintained in f/8 medium (Guillard and Ryther 1962) using sterile technique until needed.

To initiate culture experiments, cells were transferred to experimental medium containing the lowest zinc ion concentration that would support adequate growth (i.e., a specific growth rate of 0.4 to 1.0 d^{-1}). The cells were acclimated for 4 to 7 d (depending on the culture growth rate) and then were inoculated into ^{65}Zn- or ^{14}C-labeled media at total cell volumes of 0.0025–0.1 μl of cells per liter of medium. The algae were grown for 8–12 cell generations, usually to near the end of the exponential phase. Total cell concentrations and volumes were measured daily in duplicate samples with a Coulter (Multisizer II) electronic particle counter in all cultures except the ^{14}C-labeled cultures in the low-light experiment (3), which were not opened until the time of cell carbon measurement. Specific growth rates of cultures were computed from linear regressions of the natural log of mean cell volume versus time for the exponential phase of growth.

Culture samples were removed for measurement of cellular Zn and C in ^{65}Zn- and ^{14}C-labeled cultures, respectively, during the exponential phase of growth, 7–11 cell divisions after inoculation (Sunda and Huntsman 1992, 1995). Subsamples were also removed for analysis of pH, Chl *a*, total cell volume, and average volume per cell in all experiments (Sunda and Huntsman 1995, 2004) and for cellular carbonic anhydrase activity in experiment 2. Total cell volume and volume per cell were measured in duplicate subsamples as described above. The cultures were sampled at low total cell volumes (a mean of 4.2 ± 2.0 μl of cell volume per liter of culture) to minimize variations in culture CO_2 and pH during the growth period prior to cell analysis. For experiments conducted under a 14-h photoperiod, the cultures were sampled for analysis 4–6 h after the start of the light period.

To determine the cellular zinc concentration, the cells were filtered onto a 2-μm pore polycarbonate filter, and ^{65}Zn activity in the cells and a culture aliquot were measured with a gamma spectrometer (Sunda and Huntsman 1992, 1995). The fraction of radiotracer in the cells was multiplied by the total metal concentration and divided by the total cell volume to yield cellular metal concentrations in units of mol (liter cell volume)$^{-1}$. These values were converted to molar metal : carbon ratios by dividing them by the cell C : volume ratio measured in parallel ^{14}C-labeled cultures (experiments 3 and 4) via liquid scintillation counting (Sunda and Huntsman 1992, 1995) or estimated from previous experimental values (experiments 1 and 2) (*see* Table 1). Cellular Zn uptake rates [μmol (mol C)$^{-1}$ d^{-1}] were computed by multiplying the cellular zinc : carbon ratios by the specific growth rate (Sunda and Huntsman 1992, 1995).

Cellular Chl *a* : C ratios were determined by an analogous procedure. Cells were filtered onto a 2-μm pore polycarbonate filter; extracted into a mixture of 45% dimethylsulfoxide, 45% acetone, and 10% water (Shoaf and Lium 1976); and measured for Chl *a* with a Turner Designs model 10-AU fluorometer using the optical settings specified by Welschmeyer (1994). The Chl *a* values were normalized to cell volume and then converted to Chl *a* : C molar ratios using the C : cell volume ratios in Table 1.

Carbonic anhydrase activity was measured in experiment 2 using a modification of the method of Husic et al. (1989). In this assay, cells from 300 ml of culture were filtered onto a 3-μm pore Nuclepore filter. The retained cells were rinsed with filtered Gulf Stream seawater and resuspended in 3 ml of ice cold 22 mmol L^{-1} Na-barbital buffer held in a 7-ml Teflon vial. The vial was placed in an ice bath (0°C), and the cells were disrupted by sonication following the addition of a drop of Triton X100. For pH 8.2 cultures at the lowest [Zn′], CA activities were expected to be low, and for these cultures a single 2.5-ml aliquot of the crude cell extract was

Table 1. Cellular zinc and carbon per liter of cell volume, specific growth rate, and volume per cell for various experimental treatments.

Experiment	Light intensity (μmol quanta m^{-2} s^{-1})	Light period (h)	pH	log [Zn′]	Cell Zn (μmol L_{cell}^{-1})	Cell carbon (mol L_{cell}^{-1})	Growth rate* (d^{-1})	Volume per cell† (fL)
1	500	14	8.2	−12.05	32.7	22‡	0.10±0.01	41±2
				−11.47	52.2	15‡	1.23±0.05	36±2
				−10.87	122	15‡	1.62±0.09	43±1
				−10.36	153	15‡	1.76±0.05	44±2
				−9.82	182	15‡	1.76±0.01	45±2
1	500	14	9.0	−11.96	37.8±0.2§	15‡	0.51±0.06	43±3
				−11.38	85.0	15‡	1.12±0.06	43±1
				−10.78	204	15‡	1.28±0.05	42±2
				−10.27	406	15‡	1.42±0.07	47±4
				−9.73	503	15‡	1.49±0.12	46±4
2	500	14	8.2	−11.82	49.2	15‡	0.64±0.01	29±1
				−11.82	40.3	15‡	0.79±0.01	36±4
				−10.87	131	15‡	1.45±0.01	36±4
				−9.82	201	15‡	1.51±0.07	37±2
2	500	14	9.0	−11.71	58.1	15‡	0.73±0.04	29±2
				−11.71	58.1	15‡	0.78±0.03	31±2
				−10.76	187	15‡	1.39±0.04	35±2
				−10.76	205	15‡	1.38±0.01	35±1
				−9.71	428	15‡	1.44±0.03	35±1
				−9.71	500	15‡	1.42±0.02	35±1
3	50	14	8.2	−12.32	28.4±0.4§	22.4±0.8§	0.11±0.04	41±6
				−11.82	54.8±1.2§	14.0±0.3§	0.59±0.01	29±2
				−11.32	126±8§	14.6±1.0§	0.62±0.02	31±1
				−10.82	224±14§	14.9±0.7§	0.58±0.01	32±1
				−10.32	258±22§	14.8±1.2§	0.62±0.01	33±3
				−9.82	258±8§	13.2±0.4§	0.62±0.02	32±2
4	500	24	8.2	−11.82	53.9	18.1±1.8§	0.72±0.01	32±1
				−11.32	57.1	15.2±1.2†	1.45±0.01	42±0
				−10.82	122	14.7	2.00±0.04	39±0
				−10.32	220	15.1	2.03±0.03	41±0
				−9.82	254	15.6	2.04±0.01	41±0

* Specific growth rate±regression slope standard error for ^{65}Zn-labeled cultures (experiments 1–3). Mean rate±range for ^{65}Zn- and ^{14}C-labeled cultures in experiment 4.
† Mean±SD for n=3 or 4.
‡ Estimate based on previous measurements (Sunda and Huntsman 2004) and values measured under continuous high light and 14 h per day of low-intensity light.
§ Mean±range for n=2.

used for each assay. For all other cultures 1.3-ml duplicate aliquots of the cell extract were each mixed with 1.2 ml of ice cold barbital buffer to yield a total volume of 2.5 ml. Each extract solution was placed in a 7-ml Teflon vial in an ice bath, and the vial was sealed with a silicone stopper through which a pH combination electrode and a syringe had been inserted. We added 1.2 ml of CO_2 saturated Milli-Q water (0°C) from the syringe, and the time required for the pH to drop from 8.0 to 7.0 was recorded. The same measurements were made in 2.5-ml control buffer samples containing no cell extract. The activity of carbonic anhydrase (A) was calculated from the formula $A = (t_c/t_s - 1) \times 10$, where t_c and t_s are the times required for the decrease in pH from 8.0 to 7.0 for the control and cell extract, respectively. The activity units per milliliter of cell suspension were divided by the total cell volume per milliliter, measured in a 50-μl aliquot of the suspension just before cell disruption. These values were then divided by the cell C : volume ratio (Table 1) to yield CA activity normalized to cell carbon.

Statistical analyses—*t*-tests for significant differences between mean values were conducted with a Microsoft Excel computer program. The same program was used for linear regression statistical analyses. A comparison of regression slopes was made using a *t*-statistic test (Sokal and Rohlf 1981).

Results and discussion

The effect of increasing pH and associated decrease in CO_2—In experiments 1 and 2 the CO_2 concentration in the culture medium was decreased from its ambient value of ~10 μmol L^{-1} to ~0.9 μmol L^{-1} by adjusting the pH from 8.2 to 9.0 via NaOH addition. Plots of specific growth rate versus the concentration of dissolved inorganic zinc species ([Zn′]) showed no substantial difference in zinc requirements at ambient and low CO_2 (pH 8.2 and 9.0) (Fig. 1A, B). This result was surprising since we had expected Zn to be more limiting at the low CO_2 concentration owing to a

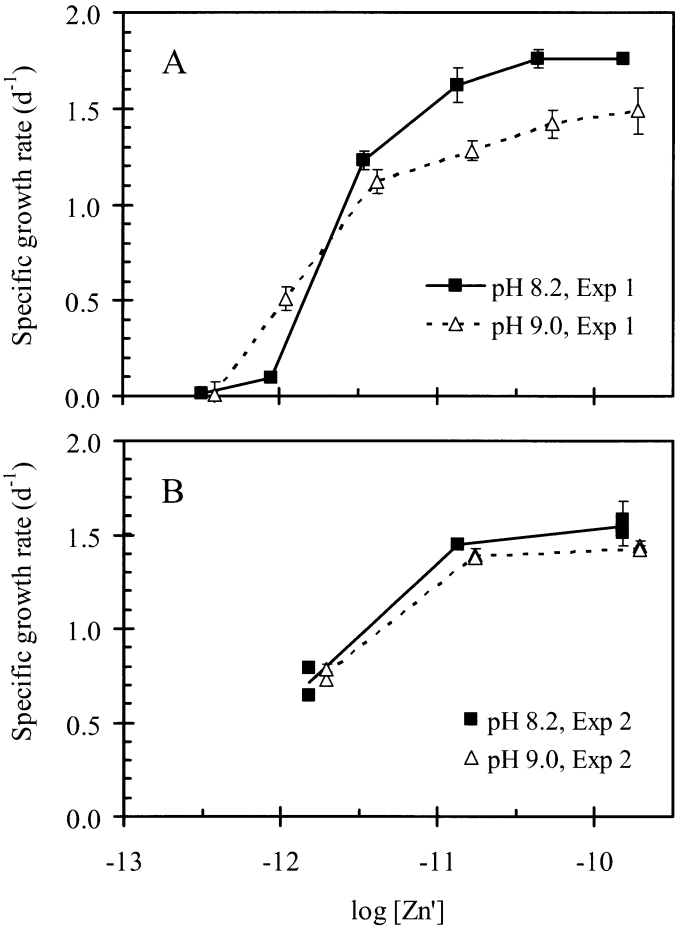

Fig. 1. Relationships between specific growth rate and Zn′ concentration at pH 8.2 and 9.0 in (A) experiment 1 and (B) experiment 2. Error bars give standard errors for linear regressions of ln cell vol. versus time.

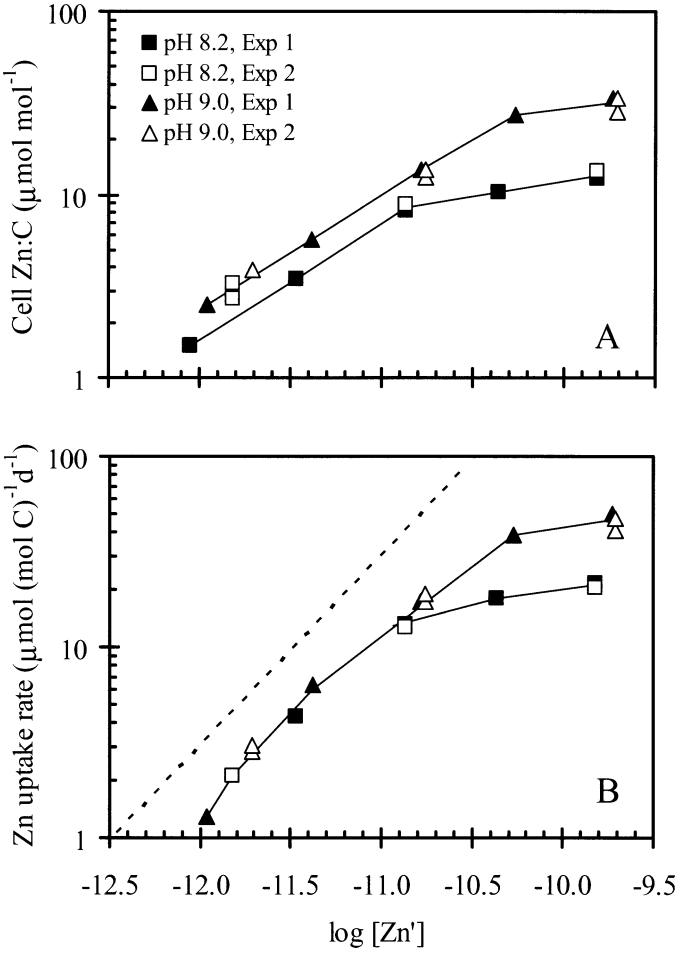

Fig. 2. Log–log relationships between (A) the cell Zn : C ratio and [Zn′] and (B) the computed zinc uptake rate and [Zn′] in the two pH/CO_2 experiments. Solid lines in panel A connect the data points in experiment 1. The dotted diagonal line in panel B gives the computed limiting rate for diffusion of Zn′ species to the cell surface as previously described (Sunda and Huntsman 1998). These calculations are based on an average cell volume per cell of 34 femtoliter (a typical value at low [Zn′], Table 1) a cellular carbon concentration of 15 mol per liter of cell volume, and a Zn′ diffusion rate constant of 7 cm^2 s^{-1}.

higher demand for carbonic anhydrase. Plots of cell Zn : C versus [Zn′] provided at least a partial answer to this puzzle. Cells grown at low CO_2 (high pH) had up to 2.5-fold higher Zn : C ratios at the same [Zn′]. The largest increases occurred at the highest [Zn′] values (Fig. 2A), where specific rates of growth and carbon fixation are highest, and thus the demand for carbonic anhydrase should also be highest.

Cellular zinc uptake rates (V_{Zn}) were computed by multiplying cellular Zn : C by the specific growth rate (μ):

$$V_{Zn} = [Zn:C]\mu \quad (1)$$

Cellular zinc uptake rates were approximately proportional to [Zn′] up to a concentration of $\sim 10^{-10.8}$ mol L^{-1} (16 pmol L^{-1}) at ambient CO_2 (pH 8.2) and up to $10^{-10.3}$ mol L^{-1} (50 pmol L^{-1}) at low CO_2 (pH 9.0) (Fig. 2B). These uptake rates approached limiting values for the diffusion of kinetically labile Zn′ species to the cell surface, as found in previous investigations with this and other phytoplankton species (Sunda and Huntsman 1992, 1995). At higher concentrations, uptake rates showed minimal further increases with increasing [Zn′]. Previous studies have shown that this reduced change in uptake with variation in [Zn′] is associated with a regulation of Zn uptake (and thus cellular Zn) by a negative-feedback up- or down-regulation of a high-affinity transport system (Sunda and Huntsman 1992, 1998). In the present experiments, the ''regulated'' Zn uptake rates are higher ($p < 0.001$, *t*-test) by $\sim$2.4-fold at low CO_2 than at ambient CO_2 concentration for equivalent [Zn′] (Fig. 2B). Thus, there appears to be an up-regulation of cellular Zn uptake rates at low CO_2 concentration. This increase, in combination with a slight (5–15%) decrease in specific growth rate (the effective rate of biodilution) resulted in a 2.7-fold increase in cellular Zn : C ratios at high [Zn′] values for cells grown at low CO_2 (Fig. 2A).

The higher ''regulated'' cellular Zn : C ratios at the low CO_2 concentration are associated with an increased growth requirement for cellular zinc (Fig. 3A,B) and for carbonic anhydrase (Fig. 4A) at high specific growth rates. The effect on cellular Zn requirement was most apparent in the first

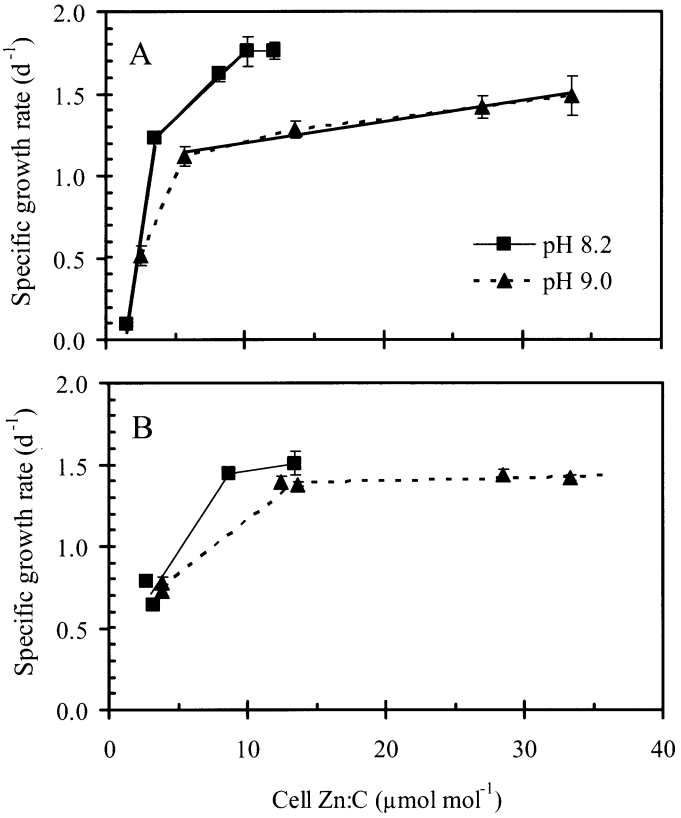

Fig. 3. Relationships between specific growth rate (±SE) and cellular Zn : C at pH 8.2 and 9.0 (ambient and low CO_2, respectively) for (A) experiment 1 and (B) experiment 2. The three straight solid heavy lines in panel A give the linear regression lines described in the text.

experiment (Fig. 3A), where the maximum specific growth rate (and thus the specific rate of net carbon fixation) was higher (1.76 d^{-1} vs. 1.51 d^{-1} at pH 8.2), which should have caused a higher demand for carbonic anhydrase. In this experiment, the relationship between specific growth rate and cell Zn : C was nearly linear at high growth rates and the slope of this relationship was greatly reduced at low CO_2 (pH 9.0) (Fig. 3A). At ambient CO_2 (pH 8.2), the slope of this relationship at high specific growth rates (1.23 to 1.76 d^{-1}) and cellular Zn : C (3.5 to 10 μmol mol^{-1}) was 80,000 ± 3,800 mol C (mol Zn)$^{-1}$ d^{-1} (±SE, $R^2 = 0.997$, $n = 3$) based on linear regression analysis. At low CO_2 (pH 9.0) the regression slope (12,800 ± 1,400 mol C [mol Zn]$^{-1}$ d^{-1}) ($R^2 = 0.976$, $n = 4$) at high growth rates (1.12 to 1.49 d^{-1}) was significantly lower ($p < 0.001$). The slope of the relationship between specific growth rate and the cellular Zn : C ratio represents the marginal Zn use efficiency, the incremental increase in net moles of carbon fixed per day per incremental increase in moles of cellular Zn. Thus, our results indicate that the marginal zinc use efficiency at low CO_2 (pH 9.0) is only 16% of that at ambient CO_2 at high specific growth rates.

Similar behavior was observed for a plot of specific growth rate versus carbonic anhydrase activity in experiment 2 (Fig. 4A). At high growth rates (0.7 to 1.5 d^{-1}), the average slope of this curve at low CO_2 (pH 9.0) was 0.55 ± 0.03 μmol C [CA activity unit]$^{-1}$ d^{-1}, only 17% of the slope observed at the ambient CO_2 concentration (3.3 ± 0.5 μmol C [CA activity unit]$^{-1}$ d^{-1}) (significant at $p = 0.002$). Moreover, the average maximum CA activity (±SE) was higher ($p < 0.001$) at low CO_2 (1,390 ± 51 vs. 291 ± 32 activity units [mmol cell C]$^{-1}$). Thus, the data in Figs. 3 and 4A indicate that disproportionately high activities of CA and high concentrations of cellular zinc are required to achieve high specific rates of carbon fixation and growth at low CO_2 concentration.

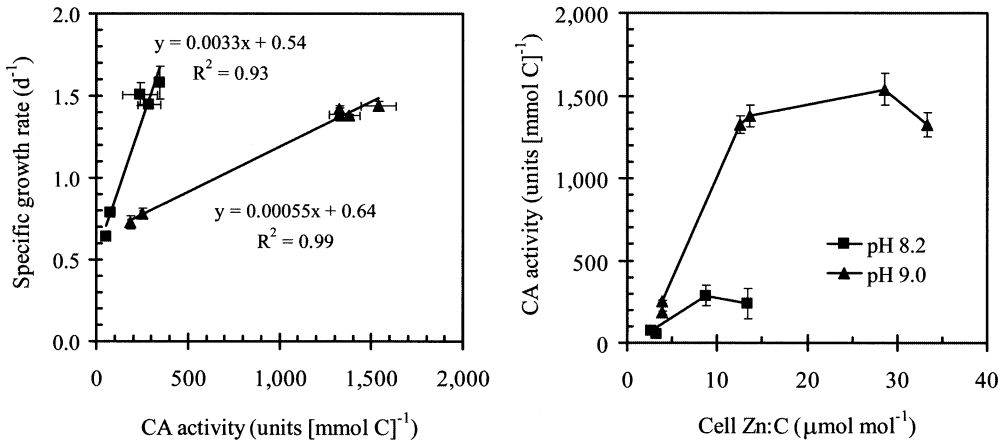

Fig. 4. Relationship (A) between specific growth rate (±SE) and carbonic anhydrase (CA) activity and (B) between cellular Zn : C and CA activity at pH 8.2 (ambient CO_2) and pH 9.0 (low CO_2) in experiment 2. Mean CA activity per mmol cell carbon (±range) is given for duplicate measurements in individual cultures, except for pH 8.2 at low [Zn′] ($10^{-9.82}$ mol L^{-1}), where only single CA analyses were made in two replicate cultures. Linear regressions were performed for each data point plotted in A ($n = 5$ for pH 8.2 and $n = 6$ for pH 9.0). The regression equations and R^2 values are shown.

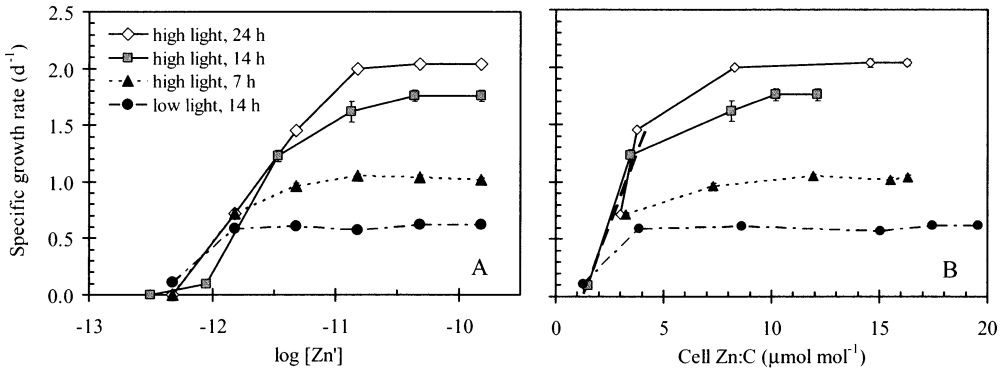

Fig. 5. Effect of photoperiod (24, 14, and 7 h d^{-1}) and light intensity (500 and 50 μmol quanta m^{-2} s^{-1}) on zinc limitation of growth rate. Specific growth rate is plotted as a function of (A) log [Zn′] and (B) cellular Zn : C ratio. Growth rates for the continuous light experiment (24-h photoperiod) are means ± range for replicate cultures (one labeled with ^{65}Zn and the other with ^{14}C). For all other treatments, error bars give ±SE for linear regressions of ln cell volume versus time for single cultures. Heavy dashed line in panel B gives the linear regression line for specific growth rate versus Zn : C under moderate to severe zinc limitation ($\mu \leq 0.7\ \mu_{max}$, $n = 6$) in the four light treatments. Results at high light and a 7-h photoperiod are taken from Sunda and Huntsman (2004).

In zinc-limited cells, CA activities decrease with decreasing cellular Zn : C and are higher at the low CO_2 concentration (pH 9.0) for a given Zn : C ratio (Fig. 4B). At low CO_2, the ratio of CA activity to cellular zinc is highest at the threshold of Zn limitation (Zn : C = 15 μmol mol^{-1}), and here most of the cellular Zn may be associated with CA. Interestingly, as cellular Zn : C increases above this threshold value, there is little further increase in growth rate or CA activity, suggesting the additional cellular zinc represents luxury consumption and storage, perhaps to allow for additional CA synthesis in the advent of further short-term decreases in CO_2.

With increasing zinc limitation, the relationships between specific growth rate and cellular Zn : C converged at ambient and low CO_2 concentrations and became indistinguishable under moderate to severe limitation (Fig. 3A, B). At growth rates ≤1.2 d^{-1} in the first experiment, the relationship between specific growth rate and cell Zn : C fit the linear relationship

$$\mu = B(\text{Zn:C} - m) \qquad (R^2 = 0.969,\ n = 3) \qquad (2)$$

The slope *B*, the marginal zinc use efficiency, had a value of 567,000 ± 101,000 mol C (mol Zn)$^{-1}$ d^{-1}) (±SE), 44 times the value observed at high growth rate and low CO_2. The term *m* (1.4 ± 0.5 μmol mol^{-1}) represents the projected maintenance Zn : C value at zero growth rate.

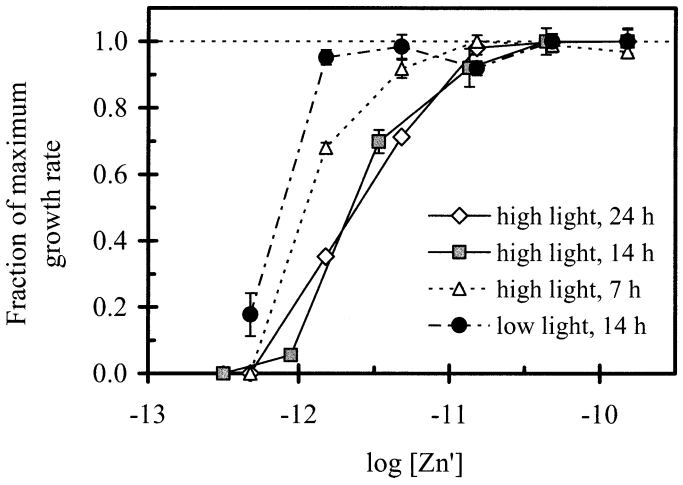

Fig. 6. Effect of photoperiod and light intensity on zinc limitation of growth rate. Growth rate is normalized to the maximum rate observed under zinc sufficiency. Error bars are computed from error values in Table 1 and Fig. 5. Data for high light and a 7-h photoperiod are from Sunda and Huntsman (2004).

Influence of light on Zn limitation of growth rate—Light intensity and photoperiod, which regulate rates of photosynthetic CO_2 fixation, also influenced cellular Zn requirements. At ambient CO_2 (pH 8.2), cells grown under saturating light (500 μmol quanta m^{-2} s^{-1}) and long photoperiods (14 h d^{-1} and continuous light) were most susceptible to zinc limitation. Cells grown under these conditions exhibited high maximum specific growth rates (1.76 d^{-1} and 2.04 d^{-1}, respectively; Fig. 5A) and showed similar progressive limitation of growth rate at [Zn′] < $10^{-10.5}$ mol L^{-1} (<30 pmol L^{-1}) (Fig. 6). A decrease in light intensity from 500 to 50 μmol quanta m^{-2} s^{-1} for cells growing under a 14-h light period decreased the maximum growth rate to 0.62 d^{-1}, but there was little change in the [Zn′] needed to achieve a given growth rate (Fig. 5A). Based on data from a previous study (Sunda and Huntsman 2004), a decrease in the photoperiod to 7 h d^{-1} had a similar but lesser effect (Fig. 5A). Consequently, zinc is less limiting under these restricted light conditions (Fig. 6). For example, under a 14-h photoperiod, a decrease in [Zn′] from $10^{-9.82}$ to $10^{-11.82}$ mol L^{-1} reduced the growth rate by ~70% at the high light intensity, but by only ~5% at the low intensity (Fig. 6).

A similar pattern was observed for relationships between specific growth rate and cellular Zn : C (Fig. 5B). At moderate to severe Zn limitation, this relationship was effectively the same for the different light treatments (Fig. 5B) as ob-

served above for ambient and low CO_2 treatments (Fig. 3). Linear regression of specific growth rate (μ) versus Zn:C at low Zn:C ($\leq$3.8 μmol mol^{-1}) fit Eq. 2 ($R^2 = 0.894$, $n = 6$). The slope B (the marginal Zn use efficiency) had a value of 497,000 $\pm$ 85,000 mol C (mol Zn)$^{-1}$ d^{-1} ($\pm$SE), while the x-intercept m (the maintenance Zn:C) was 1.3 $\pm$ 0.5 μmol mol^{-1}, similar to the values reported above ($p \geq 0.83$).

As noted previously, the relationship between specific growth rate and cell Zn:C shows much lower slopes under mild Zn limitation, indicating a substantial lowering of marginal Zn use efficiencies at higher growth rates. At saturating light, a decrease in photoperiod from 24 to 14 h d^{-1} in the present study, and to 7 h d^{-1} in a previous study (Sunda and Huntsman 2004), resulted in a progressive decrease in the marginal zinc use efficiency slope; consequently higher cellular Zn:C ratios were needed to achieve a given growth rate (Fig. 5B). For example, a cellular Zn:C of 5.1 μmol mol^{-1} is needed to achieve a specific growth rate of 1.62 d^{-1} under continuous light, but a 59% higher value (8.1 μmol mol^{-1}) is needed to achieve the same growth rate under a 14-h photoperiod. This increase in growth requirement for cellular zinc with decreasing photoperiod is consistent with an increase in specific C-fixation rate, resulting from an algal photoacclimation to shortened day length, previously observed in this and other algal species (Hobson et al. 1985; Sunda and Huntsman 2004). Such an increase in photosynthetic C-fixation rate should increase the metabolic demand for carbonic anhydrase and, thus, for cellular zinc.

We can estimate variations in specific rates of photosynthetic C fixation during the day for the three photoperiods by dividing the specific growth rate (which equals the net daily specific rate of C fixation) by the daily hours of light. For Zn-sufficient cells growing at their maximum rates, these estimated net hourly rates are 0.085, 0.126, and 0.149 mol C fixed (mol C)$^{-1}$ h^{-1} for cells grown at high light intensity under photoperiods of 24, 14, and 7 h d^{-1}. These rates equal the daytime net specific C-fixation rate minus nighttime loss of carbon due to respiration and release of dissolved organic carbon. For *T. pseudonana* growing under daily photoperiods of 7 and 14 h, these nighttime losses are ~20% of cellular fixed carbon (Sunda and Huntsman 2004). Adjusting for this loss, we estimate net daytime specific C-fixation rates of 0.157 and 0.186 h^{-1}, respectively, for cells growing under 14- and 7-h photoperiods, rates that are 1.8-fold and 2.2-fold higher than the rate (0.085 h^{-1}) under continuous light. From similar calculations, we estimate a net daytime C-fixation rate of only 0.055 h^{-1} for cells growing under a 14-h photoperiod at the low light intensity. Because of their low specific C-fixation rate, these light-limited cells should have a low metabolic requirement for carbonic anhydrase and other zinc proteins needed to support biosynthesis, largely explaining the extremely low threshold Zn:C (2–4 μmol mol^{-1}) required for maximum growth rate (Fig. 5B). By contrast, a Zn:C ratio of 10 μmol mol^{-1} is needed to support maximum growth at high light for the same photoperiod.

Not only is the specific growth rate (μ) controlled by the cellular Zn:C ratio, but the cellular Zn:C is inversely related to growth rate, as can be seen from a mathematical rearrangement of Eq. 1:

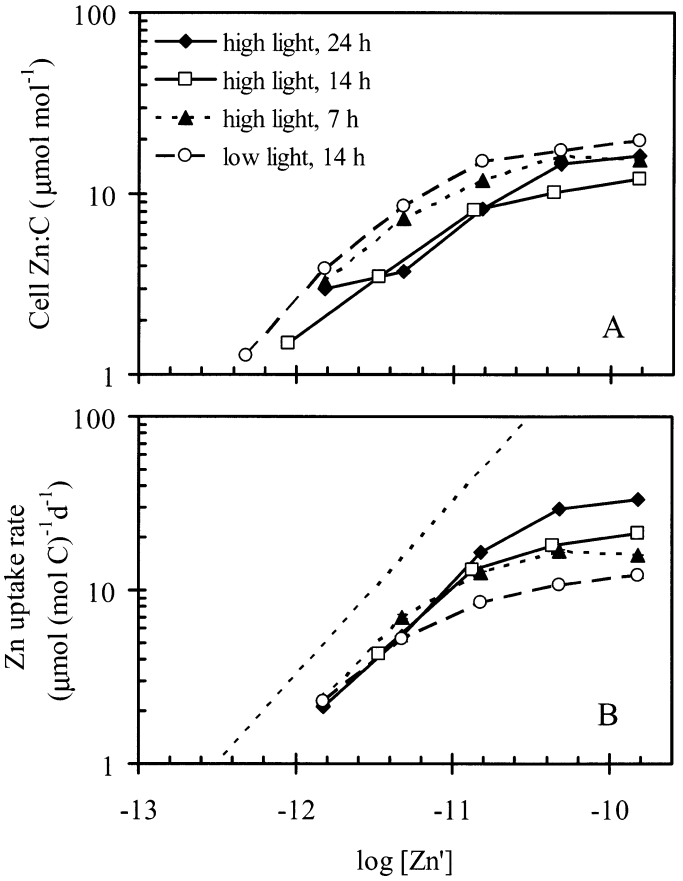

Fig. 7. Effect of photoperiod and light intensity on (A) cellular Zn:C ratios and (B) carbon-specific cellular zinc uptake rates. Data for high light and a 7-h photoperiod are taken from Sunda and Huntsman (2004).

$$\text{Cell[Zn:C]} = V_{Zn}/\mu \quad (3)$$

where V_{Zn} is the daily cellular Zn uptake rate normalized to cell carbon under steady state conditions. Such steady state conditions would have occurred in an exact sense only in our exponentially growing cultures under continuous light conditions. For the remaining cultures, which were grown under light/dark cycles, large variations in net C-fixation rates between day and night would have resulted in diel variations in cell Zn:C and specific growth rate (Sunda and Huntsman 2004). Nevertheless, the above equation does apply for average daily values of cell Zn:C, V_{Zn}, and μ. Because these average values do not change from one day to the next in our exponentially growing cultures, they can be considered to be at steady state.

As a result of the inverse relation between cellular zinc and specific growth rate, cells growing at low rates at low light intensity or shortened photoperiod generally had higher Zn:C ratios (Fig. 7A). This effect was most pronounced near the threshold of zinc limitation ([Zn′] ~ $10^{-11.3}$ mol L^{-1}) where there were large differences in growth rate (Fig. 6A), but Zn uptake rates were similar due to physical control by diffusion (Fig. 7B). The high cellular Zn:C provides an additional reason why zinc was less limiting under conditions of low light intensity or short photoperiod (Fig. 6). Under

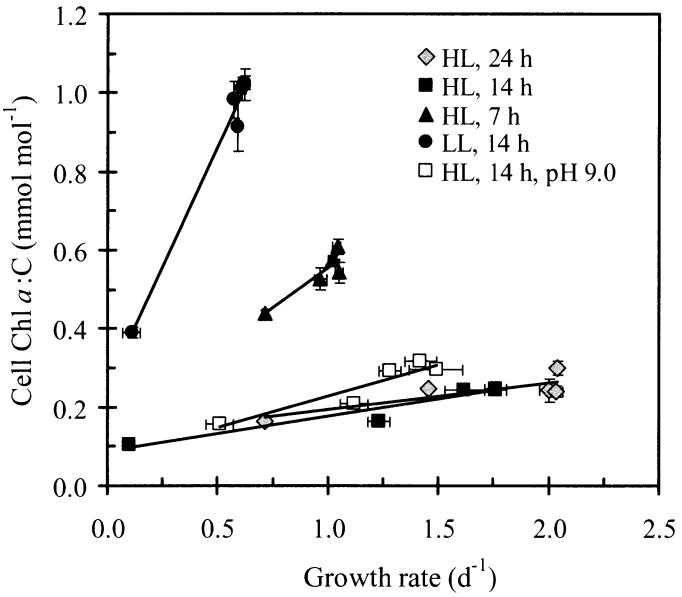

Fig. 8. Relationship between the Chl *a*:C ratio and specific growth rate under zinc sufficiency and limitation. Data are plotted for high light (HL, 500 μmol quanta m^{-2} s^{-1}), ambient CO_2 (pH 8.2), and photoperiods of 24, 14, and 7 h d^{-1}; low light (50 μmol quanta m^{-2} s^{-1}), pH 8.2, and a 14-h photoperiod; and high light, low CO_2 (pH 9.0), and a 14-h photoperiod. Growth rate errors are as defined in Figs. 1 and 5. For the low light and very short (7 h) photoperiod treatments, mean Chl *a* (±SE, $n = 4$) is plotted based on data from replicate ^{65}Zn- and ^{14}C-labeled cultures. For the continuous light treatment, mean Chl *a* (±SE, $n = 2$) is given for measurements in replicate ^{65}Zn- and ^{14}C-labeled cultures. For the 14-h treatments at high light intensity (pH 8.2 and 9.0), only single Chl *a* measurements are plotted. Linear regression lines are shown. Data for the 7-h photoperiod treatment are taken from Sunda and Huntsman (2004).

low light intensity, not only is less metabolic zinc needed to support the lower growth rate, but the cells contain higher Zn:C ratios because of the inverse relation between cellular Zn and specific growth rate (Eq. 3).

Effects on Chl a:*C ratios*—Chl *a*:C ratios in Zn-sufficient cells increased with decreasing light intensity ($p < 0.001$) and CO_2 concentration ($p = 0.03$), but there was no significant difference for cells grown under continuous light and under a 14 h d^{-1} photoperiod ($p = 0.31$) (Fig. 8). However, a comparison with previous results (Sunda and Huntsman 2004) indicates that a further decrease in the light period to 7 h d^{-1} significantly increases Chl *a*:C ratio under zinc sufficiency ($p < 0.001$) (Fig. 8). The increase in Chl *a*:C ratios with decreasing light intensity or photoperiod represents well-documented algal photoacclimations to suboptimal light conditions (Hobson et al. 1985; Falkowski and Raven 1997; Sunda and Huntsman 2004). The increase in Chl *a*:C under CO_2 limitation has been observed previously in *T. pseudonana* (Bucciarelli and Sunda 2003). It may reflect the increased demand for photosynthetically derived energy needed to fuel active uptake of inorganic carbon at low CO_2 concentrations (Kaplan and Reinhold 1999).

For any given set of conditions, the cellular Chl *a*:C ratio decreased approximately linearly with specific growth rate under varying levels of zinc limitation (Fig. 8; Table 2). The decrease in Chl *a*:C ratios under zinc limitation is unlikely to result from a specific metabolic requirement for zinc in Chl *a* synthesis, since no such role for Zn has been identified. Rather, because cells must maintain a balance among light harvesting, photosynthetic C fixation, and biosynthesis, they down-regulate their Chl *a* content with decreasing specific rates of C fixation under any given set of light conditions and CO_2 concentration. This occurs irrespective of whether these reduced rates are caused by a limiting supply of specific nutrients (N, Fe, or Zn) (Sakshaug et al. 1989; Kana et al. 1997; Sunda and Huntsman 2004) or by other factors such as low temperature.

The complex shapes of zinc limitation curves—Zinc is found in a diverse array of cellular proteins that serve in many different metabolic functions. Thus it is perhaps not surprising that curves for Zn-limited specific growth rate versus cellular Zn:C have varied shapes and that these curves respond in a complex fashion to changes in CO_2 concentration and photosynthetic C-fixation rate. Under severe zinc limitation, the relationship between specific growth rate and cell Zn:C is steep (indicating a high marginal zinc use efficiency) and is insensitive to changes in CO_2 availability or light-driven changes in the specific rate of carbon fixation. Thus, under these conditions the biochemical demand for zinc is unlikely to be primarily linked to carbonic anhydrase, but rather to other zinc proteins such as those involved in DNA replication and transcription.

At high specific growth rates there is a disproportionate increase in the cellular requirement for carbonic anhydrase and zinc, which is accentuated at low CO_2 concentration.

Table 2. Slopes, intercepts, and statistics for linear regressions of cell Chl *a*:C (mmol mol^{-1}) versus specific growth rate (d^{-1}) plotted in Fig. 8.

Experiment	Light intensity/ photoperiod*	pH	Slope±SE (mmol mol^{-1} d^{-1})	*p*	Intercept±SE (mmol mol^{-1})	*p*	R^2	*n*
1	500/14	8.2	0.09±0.01	0.02	0.09±0.02	0.02	0.922	5
1	500/14	9.0	0.16±0.04	0.02	0.07±0.04	0.24	0.863	5
3	50/14	8.2	1.22±0.08	<0.001	0.25±0.04	0.004	0.984	6
4	500/24	8.2	0.07±0.03	0.07	0.12±0.02	0.07	0.706	5
†	500/7	8.2	0.41±0.11	0.03	0.14±0.11	0.27	0.824	5

* Light intensity (μmol quanta m^{-2} s^{-1})/photoperiod (h d^{-1}).
† Results from Sunda and Huntsman (2004).

This effect appears to result directly from the role of carbonic anhydrase and thus zinc in CO_2 acquisition in algal cells (Morel et al. 1994). CA facilitates CO_2 diffusion in rapidly growing algal cells via the conversion of HCO_3^- to CO_2 at the cell surface; it also facilitates the intracellular use of HCO_3^- by catalyzing its dehydration to CO_2 within the chloroplast (Badger and Price 1994; Lane and Morel 2000).

At low specific rates of carbon fixation under severe zinc limitation or limitation by light or some other nutrient (e.g., Fe or N), most of the photosynthetic demand for CO_2 likely is met by simple diffusion of CO_2, especially for small cells such as *T. pseudonana* (cell diameter ~4 μm), whose thin diffusive boundary layer minimizes CO_2 depletion at the cell surface. Under such conditions little carbonic anhydrase may be needed. However, at high specific rates of C fixation or low CO_2 concentrations, simple diffusion may not be sufficient to meet the photosynthetic CO_2 demand. To alleviate this problem, most marine phytoplankton, including *T. pseudonana*, have evolved inducible inorganic carbon pumps (referred to as carbon concentrating mechanisms or CCMs), which are up-regulated in conjunction with CA activity under these conditions (Badger and Price 1994; Falkowski and Raven 1997; Kaplan and Reinhold 1999). Efficient CCM function appears to be dependent on CA activity, both within the periplasmic space next to the plasmalemma and in one or more intracellular compartments (Badger and Price 1994). The CCM maintains high CO_2 concentrations and high CO_2/O_2 ratios in the vicinity of Rubisco (the CO_2 fixing enzyme) in the chloroplast stroma, which compensate for this enzyme's low affinity for CO_2 and propensity to competitively react with oxygen (Falkowski and Raven 1997). Because CO_2 can freely diffuse across cellular membranes, some of the CO_2 that is pumped into the chloroplast stroma is free to diffuse back out (Raven and Johnston 1991). Thus, the CCM constitutes a leaky pump whose efficiency should decrease with increasing CO_2 gradients between the chloroplast stroma and the cell surface. Since Rubisco molecules require moderately high CO_2 concentrations to support high rates of CO_2 fixation (Falkowski and Raven 1997), the pumps are inherently most leaky, and thus least efficient, at high photosynthetic rates. It is this increased leakiness and inefficiency, we speculate, that may account for the disproportionate increase in cellular requirements for CA and Zn with increasing specific rates of growth and C fixation.

Comparison with previous results for Zn/CO_2 interactions—The increase in cellular zinc requirements with decreasing CO_2 concentration that we observed agrees with earlier findings for the coastal diatom *T. weissflogii* (Morel et al. 1994; Lane and Morel 2000). However, the magnitude of this effect appears to be larger for *T. weissflogii* than observed in our experiments. One likely reason for this greater effect is the threefold larger cell diameter in *T. weissflogii* (~12 vs. 4 μm), which restricts the diffusion both Zn′ and CO_2 to the cell surface. As a result of this effect, the zinc uptake rate in *T. weissflogii*, normalized to cell carbon, was only 11% of that observed in *T. pseudonana* at growth limiting [Zn′] (6 pmol L^{-1}) (Sunda and Huntsman 2000). Consequently, the *T. weissflogii* cells had a lower Zn : C ratio, which restricted their ability to synthesize CA. The larger cell diameter in *T. weissflogii* also would have restricted the diffusive flux of CO_2 through the surface boundary layer and thereby decreased CO_2 concentrations at the cell surface (Richardson and Stolzenbach 1995). This in turn would increase the cellular demand for periplasmic CA to increase CO_2 supply to cell surface via dehydration of bicarbonate ions. The two diffusive effects act in concert and may greatly increase colimitation by zinc and CO_2 in large rapidly growing cells.

Zinc limitation in the environment—It is clear from our data and the above discussion that zinc limitation of growth is dependent on a number of cellular and environmental factors. Zinc limitation is most likely to occur in large cells growing at high specific growth rates at low CO_2 concentrations. Decreased specific growth rates at low light intensities substantially decrease zinc limitation due to a decreased biochemical demand for Zn enzymes and to lower rates of biodilution (*see* Eq. 3). The importance of zinc in supporting high C-fixation rates at high light intensity is seen in productivity/irradiance curves from a zinc addition experiment conducted in the sub-Arctic Pacific (Crawford et al. 2003). The addition of zinc increased the rate of C fixation per gram of Chl *a* by 130% at saturating light intensity, but increased the rate by only 40% at subsaturating light.

Limitation by nutrients (e.g., N, Fe, or Mn), which also decreases specific growth rates and associated biochemical demand for zinc, should similarly decrease zinc limitation of growth at a given low Zn′ concentration. For example, limitation of *T. pseudonana* by Mn, which decreased the specific growth rate from 1.6 to 0.6 d^{-1}, was accompanied by a substantial decrease in zinc limitation at low Zn′ concentrations (Sunda and Huntsman 1998). Similar effects should occur under iron limitation, and, consequently, algal communities that are limited by iron or light or are colimited by both (e.g., the Southern Ocean) may be less susceptible to zinc limitation. Consistent with this prediction, zinc addition had no stimulatory effect on nitrate uptake by natural phytoplankton assemblages in iron-limited upwelling waters off Costa Rica and Point Conception, California (Franck et al. 2003). The addition of iron increased nitrate uptake rates in these experiments, and this effect was substantially greater when zinc was added in conjunction with iron.

Overall, the effect of iron and light on zinc limitation of growth may be hard to predict in the ocean since zinc requirements differ among algal species (Brand et al. 1983) and iron and light are known to influence algal species composition. In addition, growth limitation by light (Fig. 8A) and iron (Sunda and Huntsman 2000; Cullen et al. 2003) increases cellular Zn : C, Zn : N, and Zn : P ratios, and thus zinc may be preferentially removed relative to N and P under these conditions and may become severely depleted. Such depletion is readily observed in the sub-Arctic Pacific and Southern Ocean, two systems limited by iron and light (Martin et al. 1989, 1990; Maldonado et al. 1999; Boyd et al. 2000). This depletion may largely account for the observed zinc limitation of algal growth rate in iron-limited waters of the sub-Arctic Pacific (Coale 1991; Crawford et al. 2003); however, similar zinc limitation is yet to be observed in iron-

limited regions of the Antarctic and sub-Antarctic Oceans (Coale et al. 2003; Ellwood 2004).

Our results and those of Morel et al. (1994) indicate that algal zinc demand and associated cellular zinc concentrations can increase substantially at low CO_2 concentrations, which often occur during algal blooms in eutrophic coastal waters. Because of increased inputs of nutrients from a variety of human sources, these blooms are occurring with increasing intensity and frequency worldwide. The combination of high biomass and low CO_2 concentrations during blooms is likely to lead to substantial depletion of zinc, which potentially could cause zinc limitation of algal growth. Such simultaneous depletion of CO_2 and zinc has been observed in the southern Chesapeake Bay, a eutrophic estuary on the East Coast of the United States (Sunda et al. 1990). An intense algal bloom during late spring was accompanied by an increase in pH to 9.0 (indicating severe CO_2 depletion) and by a decrease in dissolved zinc to 3 nmol L^{-1}, one-fifth of that found at the same location the preceding winter (Sunda et al. 1990). A similar fivefold decrease in surface zinc concentrations, apparently attributable to algal uptake, was observed during the spring phytoplankton bloom in south San Francisco Bay, another eutrophic estuary (Luoma et al. 1998). The potential for zinc limitation during such blooms is hard to assess, however, because of varying levels of zinc chelation by organic ligands (Kozelka and Bruland 1998). Nonetheless, the strong depletion of both zinc and CO_2 during intense blooms in coastal waters may lead to zinc limitation of algal growth. Evidence for this is seen in a shipboard ^{14}C-incubation experiment conducted with highly eutrophic surface water from the Mississippi River discharge plume in the northern Gulf of Mexico. In that experiment, zinc additions of 12 and 40 nmol L^{-1} increased fixed carbon by 11% and 19%, respectively, after 3 d of incubation (Sunda 1987). Such zinc limitation in eutrophic coastal waters may also alter the species composition of phytoplankton communities given the large interspecific differences in zinc requirements (Brand et al. 1983).

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Received: 27 August 2004
Accepted: 8 January 2005
Amended: 5 February 2005