Polar Biol
DOI 10.1007/s00300-008-0448-6

ORIGINAL PAPER

Iron, silicate, and light co-limitation of three Southern Ocean diatom species

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Received: 21 August 2007 / Revised: 19 March 2008 / Accepted: 20 March 2008

Abstract The effect of combined iron, silicate, and light co-limitation was investigated in the three diatom species *Actinocyclus* sp. Ehrenberg, *Chaetoceros dichaeta* Ehrenberg, and *Chaetoceros debilis* Cleve, isolated from the Southern Ocean (SO). Growth of all species was co-limited by iron and silicate, reflected in a significant increase in the number of cell divisions compared to the control. Lowest relative Si uptake and drastic frustule malformation was found under iron and silicate co-limitation in *C. dichaeta*, while Si limitation in general caused cell elongation in both *Chaetoceros* species. Higher light intensities similar to SO surface conditions showed a negative impact on growth of *C. dichaeta* and *Actinocyclus* sp. and no effect on *C. debilis*. This is in contrast to the assumed light limitation of SO diatoms due to deep wind driven mixing. Our results suggest that growth and species composition of Southern Ocean diatoms is influenced by a sensitive interaction of the abiotic factors, iron, silicate, and light.

Introduction

Diatoms are an extraordinary phytoplankton class, which play a major role in global carbon fixation in all regions of the world's ocean (Sarthou et al. 2005). Especially in the SO, diatoms tend to dominate the phytoplankton community and account for as much as ~75% of the annual primary production (Nelson et al. 1995; Tréguer et al. 1995). Diatoms can build up enormous blooms and they are responsible for almost all of the silica sedimentation in the SO (Abelmann and Gersonde 1991). In addition to the macro nutrients nitrate and phosphate that are essential for the growth of all algae, diatoms also depend on the availability of silicic acid ($Si(OH)_4$) to produce their frustules.

While nitrate concentrations are high everywhere in the SO (about 25 µM; Dafner and Mordasova 1994; Tréguer and Jacques 1992) dissolved Si concentrations vary from 1 to 15 µM north of the Polar Frontal Zone (PFZ) to about 40–60 µM on the south side (Coale et al. 2004; Franck et al. 2000; Tréguer and Jacques 1992). The high Si concentrations south of the PFZ create a favorable environment for diatoms, while the low Si concentrations can limit diatom growth north of the PFZ (Brzezinski et al. 2005; Coale et al. 2004; Franck et al. 2000; Leblanc et al. 2005).

Next to Si, the trace metal iron is known to limit phytoplankton growth in general in the SO. Several in situ iron fertilization experiments in the SO proved that especially the growth of large, chain-forming diatoms was enhanced due to the addition of iron (see review in de Baar et al. 2005). Nevertheless, recent studies showed that diatoms in all size classes were able to benefit from iron fertilization (Hoffmann et al. 2006). In addition to the effect on cell growth, iron fertilization increases the maximum specific uptake rates of silicic acid in SO diatoms and enables them to fulfill their silica needs even in waters with very low Si concentrations (Brzezinski et al. 2005; de La Rocha et al. 2000; Franck et al. 2003; Franck et al. 2000). It is suggested that this is caused by an increase in the number of active Si transporters in the cell membrane (de La Rocha

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et al. 2000). Therefore, iron is often described as the proximate limiting factor for community production (Blain et al. 2002; Hutchins et al. 2001; Sedwick et al. 2002), but a co-limitation of iron and silicate is suggested for SO diatoms (Leblanc et al. 2005). It is further suggested that in addition to growth parameters, phytoplankton composition is also affected by iron and silicate and the sensitive interaction of both in the SO (Banse 1991; Hutchins et al. 2001; Leblanc et al. 2005). Iron requirements of different diatom species seem to be variable and dependent on their photosynthetic architecture as published by Strzepek and Harrison (2004). They describe that the open ocean diatom *T. oceanica* has developed low iron requirements in general, instead of the ability to adapt to low Fe concentrations. Similar mechanisms would allow diatoms of low Fe regions, such as the SO, to maintain high growth rates under low Fe because they have developed a photosynthetic apparatus that is as effective as others under high Fe.

The extremely deep mixing and the resulting low light intensities are discussed as a third main factor influencing algal growth in the SO (Mitchell et al. 1991; Nelson and Smith Jr 1991; Timmermans et al. 2001; van Oijen et al. 2004). A significant negative correlation of the wind mixed layer (WML) depth and maximum chlorophyll a concentrations (mg m^{-3}) were found in almost all in situ iron fertilization experiments (de Baar et al. 2005). Since light serves as the source of energy for photosynthesis, light intensity and duration determines the degree of photosynthetic activity. The majority of intracellular iron is required in the photosynthetic apparatus and iron limitation lowers the photosynthetic efficiency of phytoplankton (Greene et al. 1994). This suggests that phytoplankton species growing in iron limited regions suffer more from low light conditions. In other words, the cellular iron demand is enhanced under low irradiation (Raven 1990; Strzepek and Price 2000). Light limited cells of the diatom *Thalassiosira weissflogii* contained four times more Fe per C compared to controls (Strzepek and Price 2000). Based on these findings, they suggest that photoacclimation of phytoplankton could be affected by the availability of Fe and that Fe limitation could be modulated by light. Since the SO is characterized by low iron and low light conditions most of the year, phytoplankton growth is thought to be co-limited by both factors in this high nutrient low chlorophyll (HNLC) region (Timmermans et al. 2001). However, laboratory experiments suggest species-specific differences in the exact impact of iron and light co-limitation (Sunda and Huntsman 1997; Timmermans et al. 2001).

Here, we present the first study examining the effect of iron, light, and silicate co-limitation on the diatom species *Actinocyclus sp*. Ehrenberg, *Chaetoceros dichaeta* Ehrenberg, and *Chaetoceros debilis* Cleve, all isolated in the SO, in laboratory experiments. The species are important contributors to the phytoplankton community in the SO and were chosen because of their different size in order to investigate possible size-dependent reactions. Further, both *Chaetoceros* species are chain forming and we intended to compare those to a solitary species. We especially turned our attention to the interaction of these three abiotic factors on diatom growth, as well as on physiological conditions and morphologies, and the implications for the SO phytoplankton community structure.

Materials and methods

The three diatom species *Actinocyclus* sp., *Chaetoceros dichaeta*, and *Chaetoceros debilis* were isolated on board PRV "Polarstern" during the SO iron fertilization experiments EisenEx (*Actinocyclus* sp.) and EIFEX (*C. dichaeta*, and *C. debilis*). Single cells were isolated under a light microscope using small glass pipettes and rinsed at least three times in sterile filtered Antarctic seawater.

The species were grown under iron limitation in the IfM-GEOMAR culture collection at 3°C. Special care was taken to prevent contamination with iron. Every procedure was done under trace metal clean conditions in a laminar flow bench. All materials coming into contact with the cultures and/or the medium were rinsed with HCl before use. Sterile filtered Antarctic seawater enriched with macronutrients, vitamins, and EDTA-buffered trace metals (except for iron), all in f/2 concentrations, was used as culture medium. The light climate was 30 µmol photons m^{-2} s^{-1} provided by cool fluorescence tubes (OSRAM FLUORA L18 W/77 and BIOLUX 18 W/965) at a 16: 8 h light: dark cycle.

Subsamples of the same start cultures were transferred to the eight different treatments with three replicates each for every species and treatment (Table 1). The culture media for all experimental treatments was prepared as described above except for iron and silicate concentrations. Handling during the experiment was again done under trace metal clean conditions as described above. In the four low iron treatments, no iron was added to the culture media; in the four high iron treatments, 100 nM Fe was added. In these treatments, the free iron concentration was 1.55 nM Fe′ (all inorganic Fe species) estimated as in Timmermans et al. (2001). A possible iron contamination was monitored via the quantum use efficiency of PSII (F_v/F_m) throughout the experiment. It is well known that F_v/F_m values of iron-limited cells are usually below 0.3 (Greene et al. 1992) and increase within hours after iron addition. As F_v/F_m values in the low Fe treatments were low throughout the experiment (0.14–0.3; Table 2) and showed only minor differences between the replicates, we are confident that no iron contamination occurred.

Table 1 Iron and silicate concentrations and light intensities of the eight treatments

Treatment	Iron	Light (μmol photons m^{-2} s^{-1})	Silicate (μM)
LFe/LL/HSi	No addition	30	200
LFe/LL/LSi	No addition	30	20
LFe/HL/HSi	No addition	90	200
LFe/HL/LSi	No addition	90	20
HFe/LL/HSi	1.55 nM Fe′	30	200
HFe/LL/LSi	1.55 nM Fe′	30	20
HFe/HL/HSi	1.55 nM Fe′	90	200
HFe/HL/LSi	1.55 nM Fe′	90	20

The iron, silicate, and light conditions of the different treatments are shown in Table 1. The high silicate treatments were grown in 200 μM Si, which is the concentration commonly recommended in f/2 media for diatoms. This high concentration was chosen to guarantee growth throughout the experiment without Si limitation.

The ten times lower Si concentrations in the low Si treatments (20 μM Si) resulted in a NO_3^-: $Si(OH)_4$ ratio of 44, which is close to the ratio that can be found in low Si regions of the Southern Ocean, where Si concentrations are depleted to <1 μM (Brzezinski et al. 2005; Coale et al. 2004; Franck et al. 2000; Sigmon et al. 2002). Since the cell concentration in our experiments was much higher compared to the open ocean, these relatively high Si concentration already exposed the cultures to Si limitation during the experiment (see Discussion).

The light: dark cycle was kept at 16: 8 h for all treatments. All cultures were grown in 250 ml polycarbonate bottles. Before use, the bottles were cleaned with HCl three times for at least 48 h, followed by triple rinsing with Milli-Q water. The light intensity of 90 μmol photons m^{-2} s^{-1} that we chose for our high light experiments corresponds to the mean light intensity in 1–28 m depth during the whole EIFEX experiment (50°S and 2°E January to March 2004) integrated for daytime hours. The lower light intensity of 30 μmol photons m^{-2} s^{-1} corresponds to the mean light intensity in 16–42 m depth during EIFEX (Röttgers, personal communication). These data are comparable to the light levels at a similar depth measured during the EisenEx experiment. All available light levels during the EisenEx cruise showed an average of 75 μmol photons m^{-2} s^{-1} in 3–30 m depth and 25 μmol photons m^{-2} s^{-1} in 16–75 m depth (Strass, unpublished data.)

Due to the extremely different growth behavior, sampling times and experiment periods were different among the species and partly among the treatments as well. Samples for chlorophyll measurements and thus estimations of chl $cell^{-1}$ and $volume^{-1}$ were taken during exponential growth, while F_v/F_m values were determined every 2–3 days. Cell counts were taken at the beginning and the end of the experiment for *C. dichaeta* and regularly during the experiment for the other two species.

Samples for chlorophyll measurements were filtered on GF/F filters (Whatman) and immediately stored at −20°C until analysis. The frozen filters were put in polypropylene vials and 11 ml 90% acetone and glass beads (2 and 4 mm) were added. Thereafter, the closed vials were put in a cell mill for at least 5 min until the filters were completely homogenized. The vials were then centrifuged at −5°C (10 min at 5,000 rpm). The extract was carefully taken by a pipette and filled in 5 cm glass cuvettes. Extinction was measured photometrically based on Jeffrey and Humphrey (1975).

The quantum use efficiency of photosystem II (F_v/F_m) was measured using a PhytoPAM (Walz, Germany) based on Kolbowski and Schreiber (1995). Samples were dark adapted for 10 min and kept on ice directly before measurement. For determination of cell numbers, 2 ml samples were fixed with 40 μl Lugol's Solution (iodine–potassium iodide solution 1%, MERCK) and stored at 3°C in the dark until analysis. Cell counts were performed using light microscopy (Utermöhl and Axiovert 100) at different magnifications according to the size of the organisms.

The concentrations of nitrate, phosphate, and silicic acid in the media were determined at the beginning and the end of the experiment, using standard photometric methods as in Grasshoff (1999). The macronutrients nitrate and phosphate were added in f/2 concentrations (883 and 36 μM, respectively). At the end of each experiment, nitrate and phosphate were still available in surplus and the lowest concentrations measured for all treatments were 340 μM nitrate and 18 μM phosphate. We therefore can be sure that the cells did not suffer from any N and P limitation during incubation. Even in the high Si treatments with the highest growth, still about 5 μM Si were measured after the exponential growth phase in the media.

We observed that fixation with Lugol's solution broke cell chains after some months of storage. Therefore, in this study we only present data on chain length that were counted directly or within 1 week after fixation. This short time of storage did not result in any significant differences within the same treatment.

The length and width of the cells were measured under a light microscope. It has been observed that fixation with Lugol's solution can decrease the cell volume of diatoms (Montagnes et al. 1994). Therefore, the absolute cell volume measured in this study might be lower than in natural samples. Cell volume and surface was calculated as in Hillebrand et al. (1999) assuming cylindrical shape for *Actinocyclus*: $\left(\text{volume} = \pi \times \left(\frac{\text{width}}{2}\right)^2 \times \text{height and surface} = \pi \times \text{width}\right.$

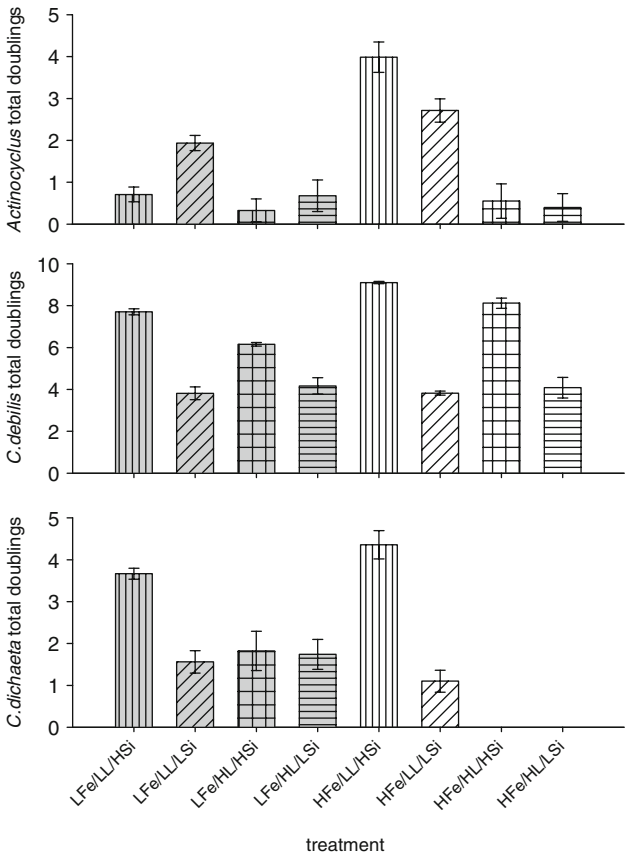

Fig. 1 Number of doublings of the three species *Actinocyclus* sp., *Chaetoceros dichaeta*, and *C. debilis* in the eight different treatments

$\times\left(\frac{\text{width}}{2}+\text{height}\right)$) and elliptic prism for the *Chaetoceros* species: $\left(\text{volume}=\frac{\pi}{4}\times a\times b\times\text{height}\right.$ and surface $=\frac{\pi}{2}\times$ $(a\times b+[a+b]\times\text{height}))$ with a being the apical section and b being the transapical section.

For statistical analysis ANOVA was used. Differences found are reported as significant in the text if $P<0.05$.

Results

Culture development

The three species tested in this study showed a different response to iron, light, and silicate availability. *Actinocyclus* was strongly influenced by light availability and showed no clear growth with less than one doubling during the experiment in all high light treatments (Fig. 1). In the low light treatments, a significant increase in the number of cell doublings was observed with higher iron availability from 0.7 ± 0.2 and 1.9 ± 0.2 doublings (treatments LFe/LL/HSi and LFe/LL/LSi, respectively) to 4.0 ± 0.4 and 2.7 ± 0.3 doublings (treatments HFe/LL/HSi and HFe/LL/LSi, respectively). Thus, the highest number of doublings was found under high iron, low light and high silicate (HFe/LL/HSi).

Growth of *Chaetoceros debilis* was clearly more influenced by silicate availability than by iron and light. Under low silicate conditions, iron and light availability had no effect on growth and there was no significant difference in the number of doublings among the low silicate treatments (4.0 ± 0.2). The number of cell doublings was always significantly higher under high silicate conditions and a further significant increase was observed under low light and high iron availability. Like *Actinocyclus*, *C. debilis* grew best under high iron, low light, and high silicate (HFe/LL/HSi) with 8.1 ± 0.2 doublings.

Like *Actinocyclus, Chaetoceros dichaeta* was strongly influenced by high light intensities, which completely suppressed growth in the high iron treatments, HFe/HL/HSi and HFe/HL/LSi. Under high light and low iron conditions, this species was able to grow, but no positive effect of increased Si availability was observed (1.8 ± 0.5 doublings in treatment LFe/HL/HSi compared to 1.7 ± 0.4 doublings in treatment LFe/HL/LSi). Under low light conditions, a significant positive effect of increased Si availability on the number of doublings was observed. This effect was again higher under high iron availability, resulting again in the highest growth in treatment HFe/LL/HSi with 4.4 ± 0.3 doublings.

To summarize these complex findings, it can be stated that all three species tested showed the highest growth under high iron, low light, and high silicate. Further, high light intensities had no positive effect on any of the species, but rather suppressed growth of *Actinocyclus* and *C. dichaeta* and hampered the positive effect of high silicate concentrations on the growth of these species.

All species were pre-cultured under low iron conditions and showed F_v/F_m values between 0.23 and 0.3 at the beginning of the experiment (Table 2) indicating iron limitation (Greene et al. 1992). In all treatments where we observed active growth, mean F_v/F_m values during the experiment were significantly higher under high iron availability. Further, mean F_v/F_m values were slightly reduced under high light intensities.

Cellular chlorophyll, cell volume, silicate uptake, and morphology

In cultures of *Actinocyclus* sp. a significant change in cellular chlorophyll concentrations was only found in treatment HFe/LL/LSi (0.16 ± 0.01 ng cell^{-1}; Fig. 2). In all other treatments the mean cellular chlorophyll concentration was 0.08 ng cell^{-1} and showed no significant difference among the treatments. In both *Chaetoceros*

Table 2 Mean and standard deviation of the F_v/F_m values at the beginning of the experiment (start) and mean values during incubation (without the start value) of the three species tested in the eight treatments

Treatment	Start *Actinocyclus*	Mean	Start *C. debilis*	Mean	Start *C. dichaeta*	Mean
LFe/LL/HSi	0.29 ± 0.03	0.22 ± 0.03	0.29 ± 0.01	0.25 ± 0.08	0.23 ± 0.02	0.28 ± 0.08
LFe/LL/LSi	0.3 ± 0.03	0.29 ± 0.05	0.28 ± 0.02	0.21 ± 0.11	0.25 ± 0.01	0.3 ± 0.1
LFe/HL/HSi	0.29 ± 0.02	0.14 ± 0.02	0.28 ± 0.01	0.17 ± 0.04	0.24 ± 0.02	0.29 ± 0.05
LFe/HL/LSi	0.29 ± 0.05	0.17 ± 0.03	0.28 ± 0.01	0.17 ± 0.08	0.24 ± 0.01	0.29 ± 0.08
HFe/LL/HSi	0.3 ± 0.01	0.39 ± 0.08	0.28 ± 0.01	0.58 ± 0.08	0.24 ± 0.01	0.53 ± 0.04
HFe/LL/LSi	0.31 ± 0.01	0.31 ± 0.08	0.28 ± 0.01	0.4 ± 0.17	0.24 ± 0.01	0.5 ± 0.04
HFe/HL/HSi	0.3 ± 0.02	0.15 ± 0.03	0.28 ± 0.01	0.48 ± 0.08	0.23 ± 0.01	0.38 ± 0.06
HFe/HL/LSi	0.28 ± 0.02	0.16 ± 0.02	0.28 ± 0.01	0.32 ± 0.05	0.24 ± 0.02	0.42 ± 0.06

species, cellular chlorophyll concentrations increased under low light conditions, low silicate, and under high iron concentrations. Like in *Actinocyclus*, the combination of these three factors in treatment HFe/LL/LSi resulted in highest cellular chlorophyll concentrations in both *Chaetoceros* species (4.9 ± 0.7 pg cell^{-1} in *C. dichaeta* and 0.45 ± 0.06 pg cell^{-1} in *C. debilis*). The high cellular chlorophyll concentrations of *Actinocyclus* and *C. debilis* are clearly visible in the microscopic pictures (Figs. 3 and 4).

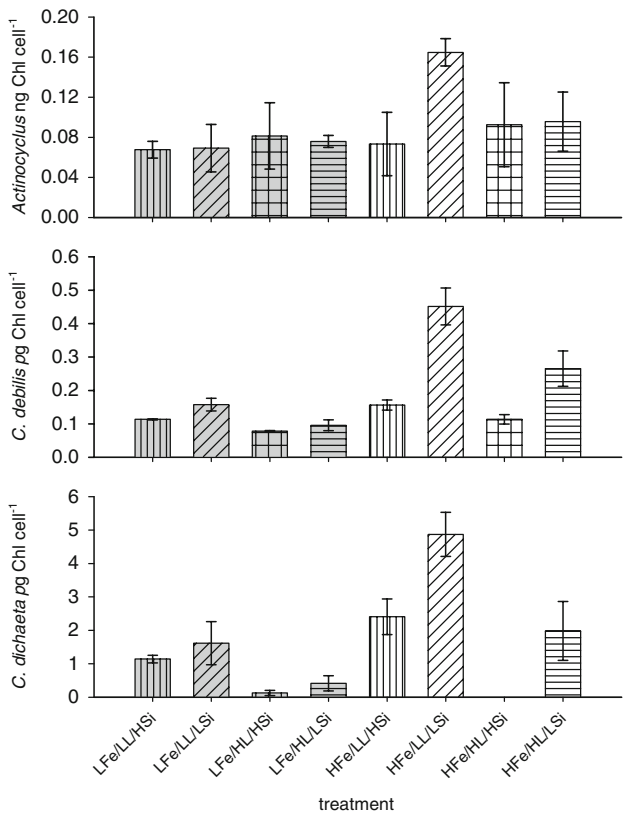

Fig. 2 Cellular chlorophyll concentrations of the three species *Actinocyclus* sp., *Chaetoceros dichaeta*, and *C. debilis* in the eight different treatments. For the *C. dichaeta* cultures, chlorophyll values for treatment HFe/HL/HSi are missing, so no chlorophyll per cell values could be estimated

The effect of nutrient limitation on cell size was again species specific (Fig. 6). In cultures of *Actinocyclus*, cell volume was between 96,297 and 152,440 µm^3 and showed no significant changes among the eight different treatments. However, this species only grew in the three treatments, LFe/LL/LSi, HFe/LL/HSi, and HFe/LL/LSi, and here cell volume was slightly lower compared to the others. In both *Chaetoceros* species, cells grown under iron limitation tended to be smaller compared to the same light and silicate conditions under high iron concentrations, respectively. However, the effect of iron on cell volume was minor and often not significant compared to the effect of silicate. In *C. dichaeta*, silicate limitation led to a significant increase in cell volume of up to 4.7 times (treatment LFe/HL/HSi and LFe/HL/LSi). In *C. debilis* cultures, cells grown under silicate limitation again showed a significantly higher increase in cell volume of almost three times. In both species, this increase in cell volume under low silicate conditions was caused by a visible elongation of cells (Figs. 4, 5, 7).

As both cellular chlorophyll concentrations and cell volume were affected by iron, silicate, and light, we determined chlorophyll concentrations per cell volume to be able to better compare the treatments (Fig. 8). In *Actinocyclus*, concentrations of chlorophyll per cell volume were three times higher in treatment HFe/LL/LSi (1.76 ± 0.41 fg µm^{-3}) compared to the other treatments that had a mean concentration of 0.63 fg µm^{-3} and showed no significant difference between each other. In both *Chaetoceros* species, chlorophyll per cell volume tended to be higher under high iron, high silicate, and low light conditions, although the differences were often not significant.

Using the reduction in Si concentrations during the experiments, we determined the Si uptake as compared to

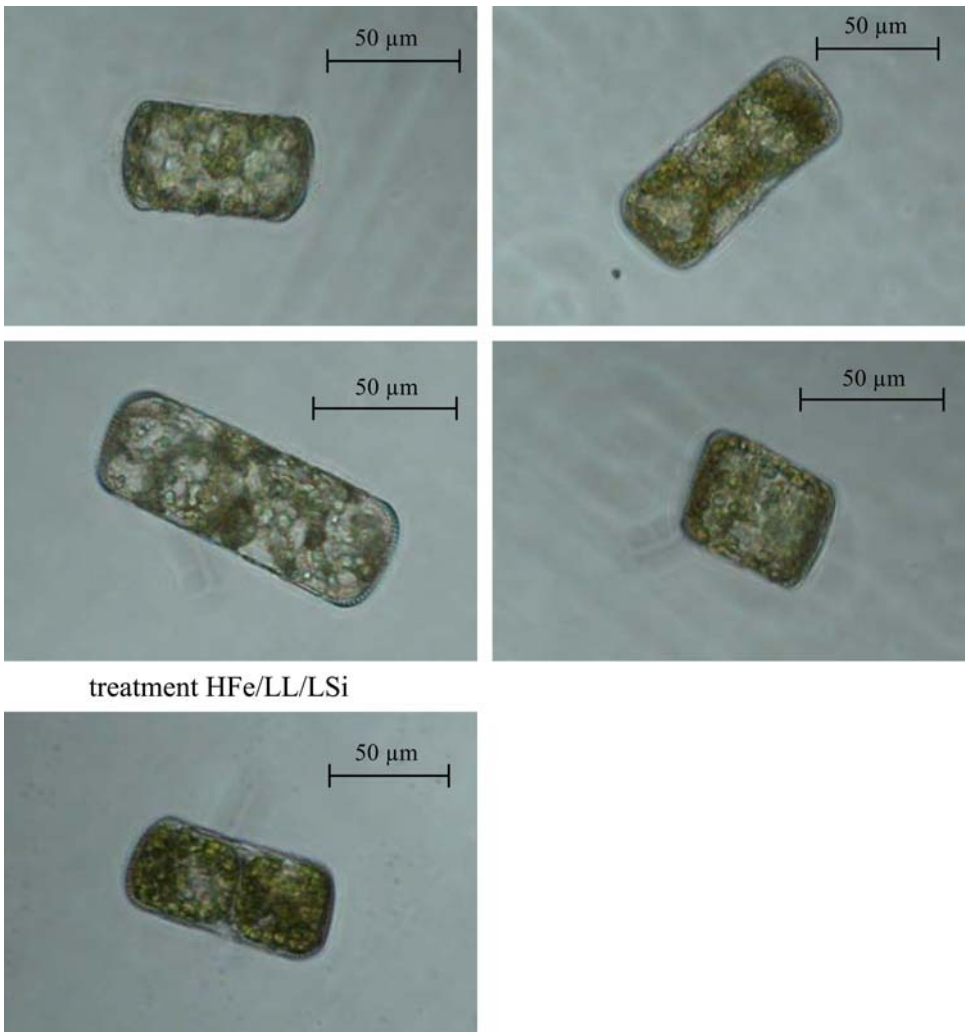

Fig. 3 Light microscopy pictures of *Actinocyclus* sp. The variance in cell size and Chl content was found in all treatments. In treatment HFe/LL/LSi (picture of dividing cell), all cells had visibly higher cellular Chl concentration

the cell surface in the different treatments (Fig. 9). This calculation was only done for the treatments where the algae grew, since no significant reduction in Si concentrations was observed in the other treatments. Therefore, it is difficult to interpret the Si uptake data for *Actinocyclus*, where only three treatments grew with more than one total doubling. In both *Chaetoceros* species, a clear and significant increase in the Si uptake per cell surface was found under low iron, but high Si conditions (LFe/LL/HSi and LFe/HL/HSi). In *C. dichaeta*, the two treatments with the lowest Si uptake per cell surface (LFe/LL/LSi: 0.9 ± 0.2 fmol μm^{-2} and LFe/HL/LSi: 0.6 ± 0.1 fmol μm^{-2}) showed a distinct frustule malformation in the light microscope (Fig. 5). In both *Chaetoceros* species, the highest Si uptake per cell surface was observed in the treatment LFe/HL/HSi with 3.8 ± 0.5 fmol μm^{-2} in *C. debilis* and 12.9 ± 5.9 fmol μm^{-2} in *C. dichaeta.*

C. dichaeta and *C. debilis* are both chain-forming diatoms. The chain length of both species was influenced by iron, light, and silicate (Fig. 10a, b). In the low iron treatments of *C. dichaeta* cultures, 70–98 % of all cells were single cells or in two-cell chains (Fig. 10a). The longest chains were found in the high iron, low light, high silicate treatment HFe/LL/HSi, where 65% of all cells were in chains of three to five cells and 19.3 % in six to ten cell chains. In all *C. debilis* cultures grown under iron limitation, almost 100 % of all cells were single cells or in two-cell chains (Fig. 10b). The increase in chain length was highest in the two high iron, high silicate treatments, HFe/HL/HSi and HFe/LL/HSi. Here, about 50% of all cells were in chains of three cells and more. Additionally, in treatment HFe/HL/HSi, up to 16 cells per chain were occasionally observed.

Discussion

The Southern Ocean is the largest HNLC region of the worlds' oceans where various factors suppress growth of primary producers despite the generally high nitrate

Fig. 4 Light microscopy pictures of *C. debilis* in the eight treatments

concentrations. The low iron concentrations in the SO are known to limit algal growth in general, while diatoms are additionally limited by low silicate concentrations north of the PFZ. The wind mixed layer depth in the SO is generally high and can reach up to about 100 m after storm events (de Baar et al. 2005). Because of these deep mixing events, the phytoplankton cells are often exposed to very low light intensities, which are thought to additionally limit the photosynthetic activity and thus growth.

In this study, we examined the effect of co-limitation of the three main parameters that may limit diatom growth in the SO: iron, silicate, and light in laboratory experiments. We are aware that laboratory experiments can only try to imitate nature and never create a truly natural environment. However, while focusing on certain key variables under controlled laboratory conditions, information about some adaptation strategies can be obtained.

The nutrient concentrations in culture media are usually much higher compared to natural conditions. This is necessary to reach sufficient biomass in a relatively small volume so that there is enough material for analysis. However, the Si concentration 20 µM used in our low Si treatments may seem very high, considering the natural concentrations. The following calculation shows that the Si

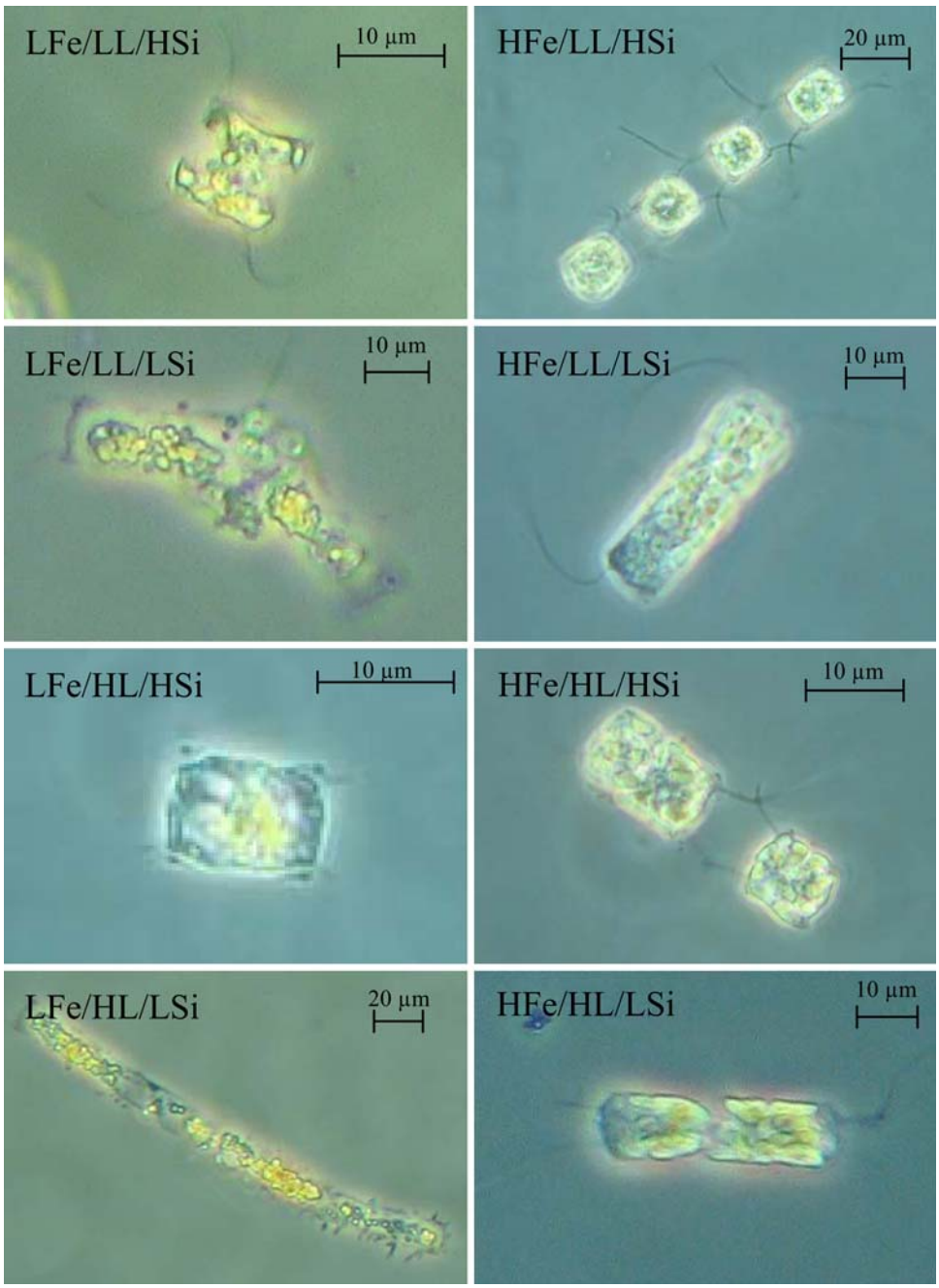

Fig. 5 Light microscopy pictures of *C. dichaeta* in the eight treatments

concentration used was low enough to expose the cells to Si limitation during the experiment:

The *C. dichaeta* strain used in this experiment has a cellular Si content of about 0.7 pmol under favorable growth conditions (Hoffmann et al. 2007). This species had a start concentration of 11,000,000 cells/l in all treatments of our experiment. Therefore, 7.7 µM Si would be taken up at the first and additional 15.4 µM Si at the second cell division assuming normal silicification. In other words, at a start concentration of 20 µM Si and a cell density of 11,000,000 cells/l, this species could not even pass two complete cell divisions under normal silicification before all Si of the growth medium is taken up (7.7 + 15.4 = 23.1 µM).

The effect of light intensity on diatom growth

Light limitation is thought to be one major reason for low phytoplankton biomass and drawdown of nutrients in the euphotic zone of the SO. Mitchell et al. (1991) modeled that under the deep mixing conditions given in the SO, only ~10 % of the available nutrients could be utilized due to

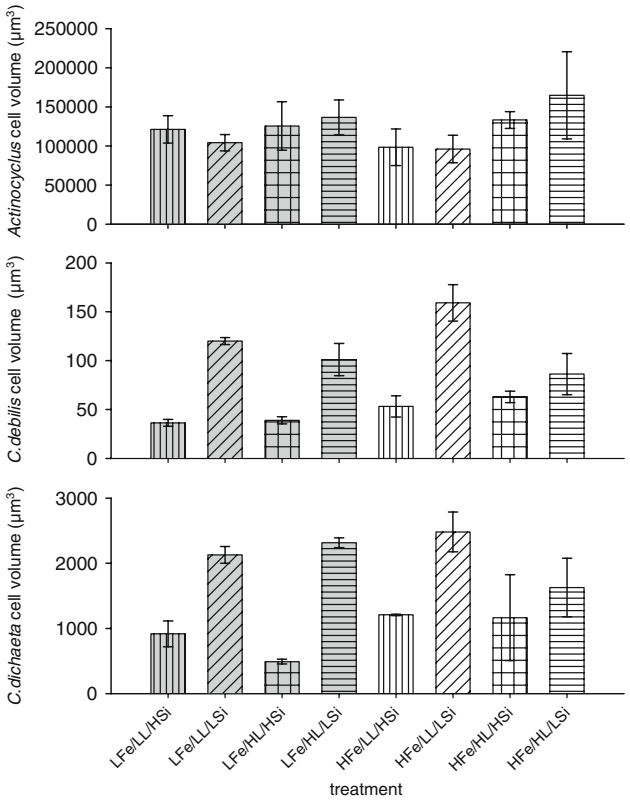

Fig. 6 Cell volume of the three species *Actinocyclus sp.*, *C. dichaeta*, and *C. debilis* in the eight different treatments

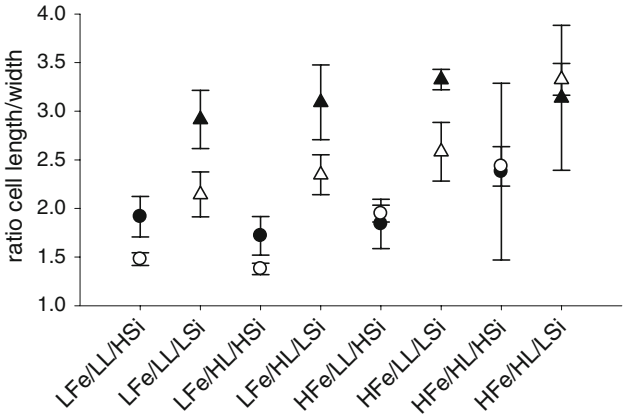

Fig. 7 Length/width ratio of the two diatom species *C. dichaeta* (*closed symbols*) and *C. debilis* (*open symbols*) in the eight different treatments. Low silicate treatments (LSi) are marked with *triangles*

light limitation. In accordance with this model, a negative correlation between WML depth and chlorophyll concentrations (mg m^{-3}) was observed in in situ iron fertilization experiments (de Baar et al. 2005). However, when integrated to mixed layer depth, chlorophyll concentrations during EIFEX were the highest compared to all other in situ iron fertilization experiments, despite the very deep mixing (Peeken unpublished data).

The importance of iron in photosynthesis stems from high concentrations in the photosystem I and II and the cytochrome b_6f complex (Raven 1990). Under low light intensities, the production of light-harvesting pigments is enhanced and thus the cellular iron requirements increase (Strzepek and Price 2000; Sunda and Huntsman 1997). In regions like the SO where iron is limited, low light intensities are therefore likely to co-limit phytoplankton growth. However, it has been described recently that the oceanic diatom species *Thalassiosira oceanica* had a much lower concentration of the iron-rich parts of the photosynthetic apparatus, photosystem I and cytochrome b_6f complex, compared to the coastal species *T. weissflogii* (Strzepek and Harrison 2004). This leads to a significant decrease in cellular iron demand, while growth and F_v/F_m stayed at a high level, comparable to those of the coastal species. Whereas the exact physiological mechanisms remain unknown so far, this apparent paradox is explained by a higher effective absorption cross-section and turnover rate of photosystem I in the open ocean species, possible in adaptation to the low natural iron concentration (Strzepek and Harrison 2004). Similar adaptation strategies could enable SO diatoms to sustain high growth under iron and light conditions that would limit other species.

In this study, we could not find a general limiting effect of low light intensity. *Actinocyclus* sp. and *C. dichaeta* were clearly not light limited grown under 30 µmol photons m^{-2} s^{-1}. This equals about the light intensity in 16–42 m depth, depending on surface radiation in the open SO during EIFEX (Röttgers, personal communication). However, in the field, phytoplankton cells are never exposed to constant light intensities, but undergo permanent changes in the light climate due to mixing and changes in weather conditions. Assuming surface irradiances between 100 and 500 µmol photons m^{-2} s^{-1}, phytoplankton cells would be exposed to mean light intensities of 30 µmol photons m^{-2} s^{-1} when constantly mixed between the surface and 44 m and more than 200 m, respectively. This assumption suggests that mixing depths of about 100 m, as commonly observed in the SO, may on average not result in a limiting light climate.

In our experiments, an increase by a factor of three to 90 µmol photons m^{-2} s^{-1} (mean light intensity in 1–28 m depth) at the same light: dark cycle suppressed growth and F_v/F_m of *Actinocyclus* under low and high iron concentrations and of *C. dichaeta* under high iron concentrations. In contrast to our findings, laboratory experiments with single species and deck incubations with natural phytoplankton assemblages suggest an iron and light co-limitation of the SO phytoplankton. Although these

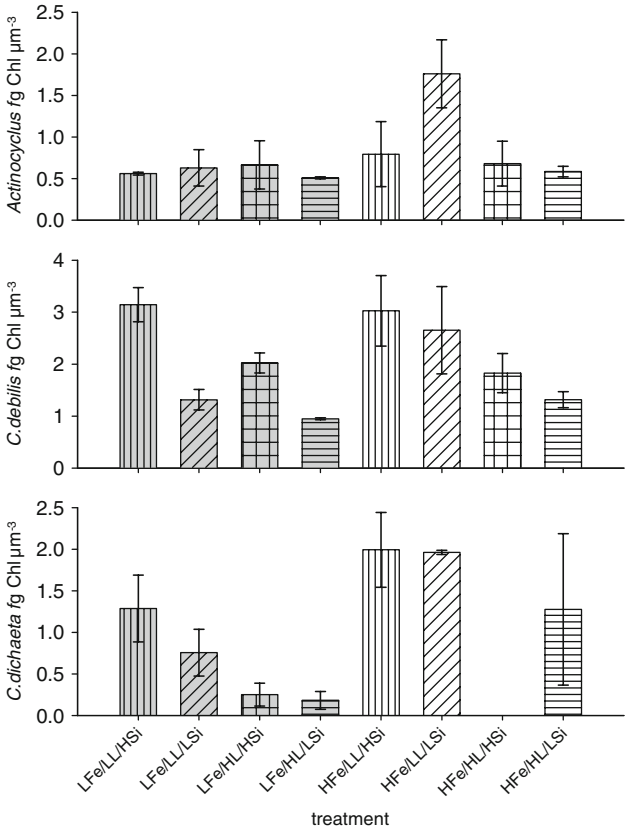

Fig. 8 Chl per cell volume of *Actinocyclus* sp., *C. dichaeta*, and *C. debilis* in the eight different treatments. For the *C. dichaeta* cultures, chlorophyll values for treatment HFe/HL/HSi are missing, so no chlorophyll per volume values could be estimated

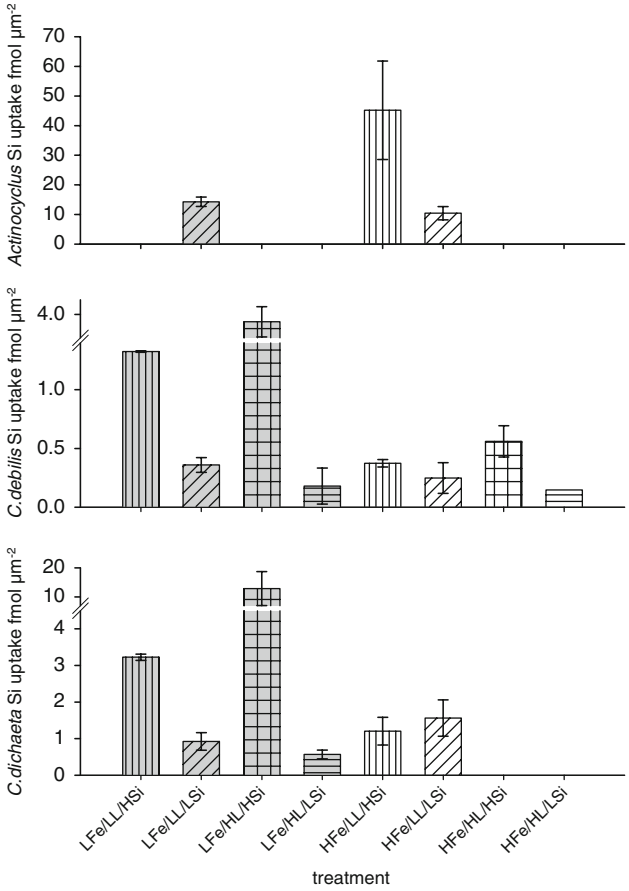

Fig. 9 Total Si uptake per cell surface of *Actinocyclus* sp., *C. dichaeta*, and *C. debilis* in the eight different treatments. No Si uptake could be estimated in the treatments where the cells did not grow

experiments are difficult to compare, as some laboratory experiments were not performed with SO phytoplankton species (Strzepek and Harrison 2004; Strzepek and Price 2000; Sunda and Huntsman 1997) and light intensities differ from 20 to about 900 µmol photons m^{-2} s^{-1} and from light : dark cycles of 12: 12 to 24: 0 h (de Baar et al. 1990; Martin et al. 1990; Sunda and Huntsman 1997; Timmermans et al. 2001), it can be summarized that smaller species are reported to be less affected by iron and light co-limitation compared to larger ones. Timmermans et al. (2001) for example report that *C. dichaeta* was only able to grow in a light: dark cycle of 20: 4 h at 80 µmol photons m^{-2} s^{-1} while no growth was detected under the same light intensity at a light : dark cycle of 12 : 12 hours. They conclude that *C. dichaeta* is iron and light co-limited under short day conditions. However, in these experiments the absolute amount of photons during one light period was 3.46 mol m^{-2} s^{-1}, which is exactly twice as much as in our low light experiments (1.73 mol m^{-2} s^{-1}). This shows that the duration of irradiance is more important than the light intensity itself. The light: dark cycle in culture experiments simulates the time of year and therefore gives no information about possible reactions to different light intensities due to changing WML depth.

Adaptation to low light in the SO was observed during the in situ iron fertilization experiment EIFEX. Although it is generally assumed that no net growth is possible below the 1% light depth, relatively high primary production of 3.4 mg C m^{-3} day^{-1} was observed at a depth with 0.1% of the surface light intensity (Peeken et al., unpublished data). The phytoplankton community of the SO is therefore able to maintain positive growth at extremely low light intensities. Similar adaptation strategies are also known for ice algae and benthic diatoms, which usually only get less than 0.1% of the surface light intensities (Admiraal 1977; Thomas and Dieckmann 2002). However, to our knowledge no such adaptation strategies are reported for pelagic diatoms in the SO.

Grown under high light intensity, the three species tested here showed very different responses. While *Actinocyclus* was not able to grow in any of the high light treatments and *C. dichaeta* only grew under high light and low iron, *C. debilis* seemed to be able to deal with higher

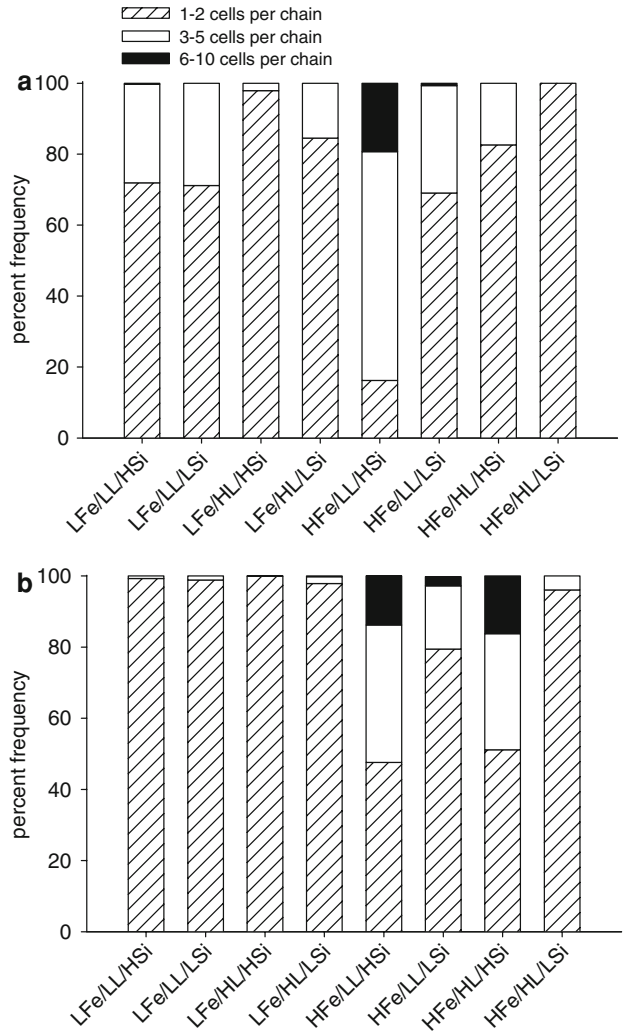

Fig. 10 Chain length of *C. dichaeta* (**a**) and *C. debilis* (**b**) in the eight different treatments

light intensities. In this species, we observed only a small decrease in the number of cell doublings under high light conditions in the high silicate treatments and no change under silicate limitation (Fig. 1). *C. debilis* is not endemic in the SO, but more or less globally distributed (Anderson et al. 2004). This means that this species has to be adapted to a variety of very different light and nutrient environments. Further, *C. debilis* may be more susceptible to grazing than larger species. Being able to sustain high growth rates under varying environmental conditions can therefore be essential to survive. Under shallow mixing conditions such "generalists" as *C. debilis* are likely to have an advantage over low light adapted species.

Several studies show that species-specific differences in the level of iron and light co-limitation exist (Sunda and Huntsman 1997; Timmermans et al. 2001). Timmermans et al. (2001) conclude from their findings that mainly larger diatoms are iron and light co-limited and that low iron and low light conditions in the SO will favor the growth of small diatoms. The reason why those cannot build up high biomasses is assumed to be due to higher grazing pressure. In contrast to Timmermans' conclusion, our findings show that the large diatoms species tested here (*Actinocyclus* sp. and *C. dichaeta*) are not light limited, but that higher light intensities have a negative effect on growth. A shallower mixing and the resulting higher irradiance would therefore not favor the growth of these larger diatom species. The observation that diatom blooms in the SO are dominated by large diatoms highlights the importance of grazing to suppress the biomass of smaller diatoms.

The effect of Fe and Si limitation on diatom growth

In situ iron fertilization in the SO showed that community growth was more enhanced by iron addition in high silicate waters compared to low silicate waters (Coale et al. 2004; Coale et al. 2003; Leblanc et al. 2005). Similar to these findings from the field, growth of all three species tested in this study was clearly co-limited by iron and silicate, as highest growth and F_v/F_m were reached in the high iron, high silicate, low light treatment.

Nutrient requirements are generally assumed to be linked to cell size as uptake rates are dependent on the surface to volume ratios (Chisholm 1992; Morel et al. 1991). Thus, smaller species are less affected by nutrient limitation compared to larger species. In this context Timmermans et al. (2001) describe that growth of the small Antarctic diatom *C. brevis* was not limited by low iron concentrations, while the larger *C. dichaeta* was. In contrast to that, a positive effect of iron on the growth of the small diatom species *Fragilariopsis cylindrus*, *Cylindrotheca closterium*, *Chaetoceros* sp., and one unidentified pennate diatom during the in situ iron fertilization experiment EIFEX was described by Hoffmann et al. (2006). Sedwick et al. (2002) suggested that larger diatom species might be more silicate limited and that these species therefore bloom in high silicate waters when iron becomes available. In agreement to this, enhanced growth of small pennate diatoms with iron addition in high and low silicate waters is described by Hutchins et al. (2001). They assume that these small, lightly silicified species are highly adapted to low Si growth conditions. However, our results demonstrate that there are small species that do not react in this way. The negative effect of silicate limitation on growth was highest in *C. debilis*, the smallest species, and lowest in *Actinocyclus*, the largest species tested. Larger, strongly silicified species have a higher amount of silicate per cell surface (Fig. 9) and in absolute numbers more silicate is needed to build up new frustules. However, relative to cell volume, the amount of silicate can be even higher in small

species. In our experiment, the largest species tested (*Actinocyclus*) had a roughly ten times higher Si uptake relative to cell surface compared to the two *Chaetoceros* species, indicating a much higher frustule silicification. However, relative to cell volume, the Si uptake was very similar between the three species (1.3–5.7 fmol μm^{-3} in *Actinocyclus*, 0.15–4.2 in *C. debilis*, and 0.3–9.3 in *C. dichaeta* (data not shown)). Thus, especially in combination with high growth rates, small species may be limited earlier by low silicate concentrations than slow-growing larger species. Further, the extent of Fe and Si co-limitation on growth was again higher in the smallest species and maximum cell numbers were 36 times (*C. debilis*), 7 times (*C. dichaeta*), and 3 times (*Actinocyclus*) lower under Fe and Si co-limitation compared to Fe and Si replete conditions. Thus, our data suggest that the extent of iron and silicate co-limitation is not only dependent on cell size. The differences between the species tested here and others reported in the literature suggest that the influence of nutrient co-limitation in the SO is very complex.

Possible explanations for these observations are differences in the physiological adaptations to nutrient limitation, such as the number and activity of membrane transport proteins that might compensate the effect of cell size. It is generally accepted that iron limitation decreases the maximum specific uptake rate (V_{max}) for silicic acid in marine diatoms, while absolute values of V_{max} differ among species (De La Rocha et al. 2000; Franck et al. 2003; Leynaert et al. 2004). This is explained by a decrease in the number of active silicate transporters in the cell membrane under iron stress (De La Rocha et al. 2000). Alternatively, it is suggested that as Si uptake in marine diatoms is linked to aerobic respiration, iron limitation decreases the electron transport efficiency of the iron-rich respiratory chain and thus causes a decrease in V_{max} (Franck et al. 2003). Thus, iron limitation decreases the capacity for silicic acid uptake in marine diatoms. This may be of less meaning in the high silicate regions of the SO, but north of the PFZ iron limited diatoms will be co-limited even faster by the low silicate availability. In this study, we could not find a positive effect of iron addition under silicate limitation on the growth of all three species tested. However, in *C. dichaeta*, lowest Si uptake relative to cell surface and visible frustule malformation was only observed under iron and silicate co-limitation. Under low silicate and high iron conditions, Si uptake was about two times higher (Fig. 9). Here, cells were elongated, but frustules showed no visible malformation (Fig. 5).

Brzezinski et al. (2005) hypothesize that diatom growth rates are limited by iron, while biogenic silica production rates and cellular silicon content may be controlled by a combined influence of both iron and silicate. Our data show that iron and silicate both have a direct influence on diatom growth. Under silicate limitation, both *Chaetoceros* species tested seemed to have problems reaching their intracellular silicate concentration needed for cell division. This is supported by the observation that cell volume is significantly higher in all low silicate treatments.

Besides the general decrease in V_{max}, the half saturation constant for silicic acid uptake (K_{Si}) is extremely different among diatom species and shows no collective trend under iron limitation (De La Rocha et al. 2000; Franck et al. 2003; Leynaert et al. 2004). This suggests that while iron may have an effect on the number of active Si transporters, their affinity for silicic acid is not Fe dependent and represents species-specific properties (De La Rocha et al. 2000). Brzezinski et al. (2005) reported a decrease in K_{Si} during the in situ iron fertilization experiment SOFEX in the low Si waters of the north patch. They suggested that either iron lowers the half saturation constants for silicic acid of individual species or causes a species shift that favors diatoms with higher Si affinity. In addition to the effect of the surface to volume ratio on nutrient uptake rates, species-specific adaptation mechanisms such as the amount and activity of transport proteins in the cell membrane may have an important impact on iron and silicate uptake rates and therefore determine the level of iron and silicate co-limitation in SO diatoms.

The observation that cells of both *Chaetoceros* species were elongated under silicate limitation is similar to that reported by Harrison et al. (1977). This suggests that low silicate concentrations not only influence the build-up of new frustule material, but also the mechanism of cell division itself. The cell cycle is classically divided in four phases: G1, S, G2 and M. While DNA is replicated during the S phase and mitosis and cell division take place in the M phase, G1 and G2 refer to "gaps" in between those processes. During these "gaps", most of the cell growth takes place (Martin-Jézéquel et al. 2000). Silicon uptake and the formation of new frustules by diatoms are non-continuous processes that are confined to the G2 phase (Brzezinski 1992; Brzezinski et al. 1990). It is described in the literature that nutrient limitation in general and resulting low growth rates lead to elongated G1, G2 and M phases and thus increased total silicate uptake (Claquin et al. 2002). However, cells grown under Si limitation may not be able to reach a certain intracellular silicate concentration. They may remain in the G1 and G2 phases and therefore do not enter the M phase and do not divide. We can only speculate what causes the extreme elongation of the cells under Si limitation (Figs. 4, 5, 7), but they may have some kind of regulatory process that stops them from dividing until they have collected a minimum amount of silicate as the new frustules may otherwise be too fragile. However, we observed that the cells continued building up plasma and that the girdle band continued growing, which

resulted in the elongated shape. Similar morphological changes are described for diatoms under silicate limitation in the field and in laboratory experiments (Harrison et al. 1977; Paasche and Østergren 1980) and are also explained by continued cell growth while cell division is blocked.

Conclusion

In conclusion we suggest that the importance of light limitation in the SO is overestimated. Our results further indicate that iron fertilization under high silicate conditions supports the growth of large and small diatoms. The general observation that large diatoms bloom in the SO when iron becomes available (see review in de Baar et al. 2005; Hoffmann et al. 2006) shows that other factors such as grazing determine species succession in an effective way. Of the species tested in this study, *C. debilis* seems to be able to adapt best to changing environmental conditions and maintain favorable growth rates. These findings in our laboratory experiments are supported by field observations from the subArctic Pacific Ocean. It has been shown that *C. debilis* is able to exceed the growth of other species in the field and becomes the dominant species after in situ iron fertilization (Tsuda et al. 2003). The growth of *C. dichaeta* and especially *Actinocyclus* was much more affected by the availability of iron, light, and silicate. However, growth of all species showed to be co-limited by iron and silicate. If nutrient availability would be the only limiting factor for growth, we would expect small "generalists" as *C. debilis* to dominate in the Southern Ocean.

Acknowledgments We thank Jesco Peschutter and Wiebke Schmidt for their help in cell counting, as well as Eike Breitbarth and Peter Croot for helpful comments and discussions. We also would like to thank the two anonymous reviewers for their constructive critics, which have remarkably improved the clarity of the manuscript. This research was funded by the German Research Foundation (DFG) grant PE_565_5.

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