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To cite this version:

Brivaëla Moriceau, Michael Garvey, Uta Passow, Olivier Ragueneau. Evidence for reduced biogenic silica dissolution rates in diatom aggregates. Marine Ecology Progress Series, 2007, 333, pp.129-142. 10.3354/meps333129 . hal-00381254

HAL Id: hal-00381254

https://hal.univ-brest.fr/hal-00381254v1

Submitted on 23 Jun 2009

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Evidence for reduced biogenic silica dissolution rates in diatom aggregates

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Running head: Biogenic silica dissolution in diatom aggregates

ABSTRACT

Because aggregated diatoms sink rapidly through the water column leaving little time for dissolution, aggregation influences the balance between recycling of biogenic silica ($BSiO_2$) and its sedimentation and preservation at the seafloor. Additionally, aggregation may directly impact dissolution rates of opal. Laboratory experiments were conducted to investigate the influence of aggregation on $BSiO_2$ dissolution rates using three different batch cultures of diatoms, *Chaetoceros decipiens*, *Skeletonema costatum*, and *Thalassiosira weissflogii*. Specific dissolution rates of biogenic silica ($BSiO_2$) of aggregated and freely suspended diatoms were compared. Additionally the influence of the dissolved silicon (DSi) concentration in the pore water of aggregates, of the viability of diatoms, the concentration of transparent exopolymer particles (TEP) and of bacteria on $BSiO_2$ dissolution rates was determined. Initial specific dissolution rates of diatom frustules were significantly lower for aggregated diatoms (2.9 % d^{-1}) than for freely suspended diatoms (6.6 % d^{-1}). Lower specific dissolution rates in aggregates were attributed to elevated DSi concentrations in aggregate pore water (maximum 230 vs. 20 µmol l^{-1}) and to the fact that aggregated diatoms remained viable for longer than freely suspended diatoms. Specific $BSiO_2$ dissolution was significantly correlated to viability of cells independent of treatment. Bacterial concentrations in both treatments appeared high enough, so that after cell death the coating protecting the silica frustule was degraded without measurable delay. The TEP content of aggregates

appeared to affect dissolution rates, possibly by retaining solutes within aggregates.

KEY WORDS: Diatom aggregate – biogenic silica dissolution – silica recycling – marine snow – viability – aggregate pore water

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INTRODUCTION

In many areas of the ocean, diatoms are the most important primary producers; they commonly form the basis of productive food webs (Silver et al. 1978, Cushing 1989, Thornton 2002) and they are responsible for a large fraction of the vertical export of carbon (e.g. Goldmann 1993, Ragueneau et al. 2001). Diatoms require dissolved silicon (DSi) to form their frustules. Intense recycling of this biogenic silica ($BSiO_2$) occurs within the mixed layer with approximately 50% of the biogenic silica produced in the euphotic zone dissolving in the upper 100 m of the water column (Nelson et al. 1995, Ragueneau et al. 2000, Ragueneau et al. 2002). Diatoms excrete dissolved polysaccharides that abiotically form transparent exopolymer particles (TEP) (Passow 2000) and enable the formation of these large aggregates (Passow et al. 1994, Passow & Alldredge 1995a). A large fraction of a diatom bloom may thus aggregate into marine snow and be rapidly transported to depth (Graf 1992, Smith et al. 1996, Thornton 2002) as aggregation increases the sinking velocity of diatoms by two orders of magnitude (50-100 m d^{-1}; Alldredge & Gotschalk 1988, Alldredge & Silver 1988).

Grazing by zooplankton and the production of silica-rich fecal pellets with a sinking velocity 30-fold greater than that of single cells (12-77 m d^{-1}; Wassmann et al. 1999, Turner 2002) also result in the rapid transport of diatoms to depth. This transport of diatoms to depths shifts the dissolution of $BSiO_2$ to below the euphotic zone, strengthening the silica pump described by Dugdale et al. (1995). The newly recycled DSi must be transported back to the euphotic zone before it can support additional diatom production.

Very little is known about the processes of the silica cycle that occur below the

euphotic zone. Our goal within the European Program ORFOIS (ORigin and Fate of biogenic particle fluxes in the Ocean and their Interactions with the atmospheric CO_2 concentration as well as the marine Sediment), has been to improve parameterization of the siliceous component of the particle fluxes through the water column. In particular we have been interested in quantifying the dissolution rates of biogenic silica contained within sinking aggregates, as these particles are a major component of the downward flux (Turner 2002, Thornton 2002).

Rates of $BSiO_2$ dissolution depend on biological variables including bacterial number and activity (Patrick & Holding 1985, Bidle & Azam 1999, 2001), on chemical variables like DSi concentrations (Van Cappellen & Qiu 1997a, 1997b) and on physical variables like temperature (Kamatani 1982, Bidle et al. 2002). Consideration of $BSiO_2$ dissolution as it occurs below the euphotic zone needs to take into account that sinking diatom frustules at these depths are mostly contained within aggregates or fecal pellets. This encapsulation could result in enhanced rates of dissolution due to the elevated rates of microbial activities on aggregates (Smith et al. 1992). Alternatively, dissolution rates could be diminished by high concentrations of silicic acid in the pore space of the aggregates (Brzezinski et al. 1997).

Schultes et al. (submitted) and Gallinari et al. (in prep) measured $BSiO_2$ dissolution of diatom frustules embedded in copepod fecal pellets. These experiments indicated that dissolution rates of diatom cells were reduced if cells were enclosed in intact fecal pellets. The first laboratory measurements of $BSiO_2$ dissolution rates of aggregated *versus* non-aggregated diatoms also indicated that incorporation within aggregates decreased $BSiO_2$ dissolution rates, but the trends were somewhat ambiguous,

as the continuous release of diatoms from and re-incorporation into aggregates obscured the results (Passow et al. 2003). Here we present unequivocal results of laboratory measurements of $BSiO_2$ dissolution of aggregated *versus* non-aggregated cells under otherwise identical conditions. Furthermore, we address the role of TEP concentration, bacterial abundance and DSi concentration in the pore water of aggregates as well as the viability of diatoms for $BSiO_2$ dissolution rates.

I. MATERIAL AND METHODS

$BSiO_2$ dissolution rates of aggregated and non-aggregated diatoms were compared in eight experiments with a total of 18 treatments conducted with *Skeletonema costatum* (North Sea 1997), *Chaetoceros decipiens* (CCMP173) and *Thalassiosira weissflogii* (CCMP1053). These three diatom species were chosen because of their abundance in natural marine snow and their abilities to grow and to aggregate in culture. *S. costatum* and *C. decipiens* form chains whereas *T. weissflogii* doesn't. Five treatments were conducted with freely suspended cells, and 13 treatments were conducted with aggregated cells. A greater number of treatments with aggregates were conducted, because of the high variability of replicate aggregates.

Cells used in the same experiment were derived from the same batch culture to ensure that they were physiologically as identical as possible. Although all batch cultures were grown under identical conditions and used for experiments in stationary phase, cells from different experiments differed in their physiological conditions as evidenced for example by differences in the viability or TEP concentrations (see below). It is

challenging to generate both an aggregated and a freely suspended treatment from cells of the same batch culture, because when cells are in aggregation mode, they tend to aggregate under almost any circumstances. We were not able to keep cells freely suspended in experiments 1, 2 and 7; and only aggregate treatments exist from these experiments (Table 1).

Preparation of experiments

Batch cultures of the respective diatoms were grown in f/2 media made from natural seawater (Guillard & Ryther 1962, Guillard 1975), at 13°C with an irradiance of 100 µmol m^{-2} s^{-1} in a 12h: 12h light: dark cycle. Except during the growth period, no glassware was used during the experiment. When a high cell concentration was reached (2×10^5 to 4×10^5 cells/ml), at or near stationary phase (Fig 1 step 1), a part of the batch culture was incubated in a 4-litre cylindrical acrylic tank made with non-toxic glue. The diatoms were kept on a roller table in the dark at 13°C to promote the formation of aggregates (Shanks & Edmondson 1989). Speeds of roller tables (about 0.5 rpm) were such that aggregates sank continuously, without spending time lying at the bottom of the roller table. The second aliquot of the batch culture was incubated in a 1-litre polycarbonate flask, under the same environmental conditions as the aggregate treatment except that the flask was placed on a shaker table (Fig. 1 step 1) where a soft horizontal agitation kept cells in suspension without visible aggregation. The cells of both treatments (aggregated and freely suspended cells) were incubated for two days to one week until more than 100 large aggregates of approximately the same size had formed in the aggregate treatment. At this time the size of aggregates also stopped increasing. The

final aggregate mean size varied from 100 to 500 mm^3 in different experiments (Table 1, column 9).

Before each $BSiO_2$ dissolution experiment, aggregated and freely suspended cells were transferred into artificial seawater containing no nutrients (Fig. 1 step 2). A 50-ml plastic pipette, with the tip cut off and smoothed, was used to isolate and transfer aggregates individually without breakage. Aggregate treatments were prepared by transferring a precise number of aggregates (60 to 150 each, depending on the size of the aggregates) into 2-litre cylindrical rolling tanks filled with artificial seawater containing no nutrients. Cells for the freely suspended cell treatments were transferred into artificial seawater containing no nutrients by allowing freely suspended cells to settle for 8 to 30 hours, until the majority of the cells resided at the bottom of the flask. The overlying media was then carefully replaced with the artificial seawater that contained no nutrients. Filtration of cells was avoided because of the risk of cell aggregation, breakage, or lyses.

Set-up of experiments

At the beginning of each dissolution experiment, 5 to 12 aggregates were isolated and their $BSiO_2$ and TEP content and bacterial and diatom numbers determined.

The $BSiO_2$ dissolution experiments were conducted in the dark at 13°C on a roller table (aggregate treatments) or a shaker table (freely suspended cell treatments). Rotation speed of roller tanks was adjusted to ensure that aggregates remained in suspension, rather than hitting container walls and varied between 0.4 and 1.0 rpm, whereas the shaker table oscillated at 2.9 Hz. Experiments were conducted in the dark to mimic sinking through deep waters, to inhibit growth of diatoms and to limit their utilization of

newly dissolved $BSiO_2$ (Blank & Sullivan 1979). Experiments lasted for 3 to 32 days each.

During each experiment, the concentration of DSi in the surrounding seawater was measured every one to two days. As part of the original media was transferred together with aggregates or freely suspended cells into the nutrient-free artificial seawater, initial DSi concentrations varied for each treatment, averaging about 20 µmol l^{-1}. Samples from rolling tanks were taken via a syringe stuck through a silicon stopper in the lid. Five milliliter samples for the determination of DSi concentration could thus be easily removed without stopping the roller table or disturbing aggregates in any way. Samples were immediately filtered through a 0.2 µm-syringe filters (Millex GP syringe driven filter unit with PES membrane; Millipore) and stored in a refrigerator until analysis. A syringe was also used to subsample freely suspended cell treatments. Sampling began as soon as aggregate sizes had stabilized after aggregate transferal; i.e. as soon as processes of aggregation and disaggregation were balanced.

Determination of initial and final $BSiO_2$ concentrations

The initial and final amounts of $BSiO_2$ were difficult to assess in aggregate treatments because variability between replicate aggregates was high (Table 2). It was therefore impossible to calculate the initial amount of $BSiO_2$ in incubated aggregates reliably from the average amount of $BSiO_2$ of the 10-20 replicate aggregates initially sampled. Thus the initial amount of $BSiO_2$ ($BSiO_{2_0}$) was calculated for each treatment (equation 1) from its final $BSiO_2$ concentration ($BSiO_{2_f}$) and the respective change in DSi concentration of the surrounding seawater ($DSi_{ssw_f} - DSi_{ssw_0}$), which represents the

amount of $BSiO_2$ dissolved during the experiment.

$$BSiO_{2_0} = BSiO_{2_f} + (DSi_{ssw_f} - DSi_{ssw_0}) \qquad \text{eq. 1}$$

The final $BSiO_2$ concentration was determined from dry weight and the ratio of $BSiO_2$ to dry weight (%$BSiO_2$ per weight) measured on aliquots of the final sample solutions. To this end, the entire content of the tanks with aggregates was shaken to break up aggregates and replicate aliquots were sampled to determine the ratio of $BSiO_2$ to the total weight in the final solution. The replication of this ratio was very good. The rest of the solution was filtered onto pre-weighed 0.4µm polycarbonate filters and weighed to calculate the final total $BSiO_2$ concentrations.

Analysis

Calculation of concentrations in aggregates: TEP, bacteria, etc. in aggregates were determined by analyzing two fractions separately; the respective concentration in the water surrounding the aggregates and the concentration in a sample containing aggregates dispersed in this surrounding seawater. Concentrations in aggregates were then calculated by difference, assuming that the volume of the surrounding seawater equaled the total volume of the aggregate slurry. Thus amounts per aggregate given here are low estimates.

Filtration for dry weight and biogenic silica: Dry weight determinations were conducted on the same filters as the determinations of $BSiO_2$. Between five and ten ml of sample containing three aggregates or five to ten ml of sample water from treatments of freely suspended cells were filtered onto pre-weighted 0.4 µm polycarbonate filters.

Dry weight: Filters were dried at 50-60°C and reweighed on a Mettler Toledo UMX2 with a precision of 0.1 µg.

Biogenic Silica ($BSiO_2$): $BSiO_2$ was determined using a variation of the method of Ragueneau & Tréguer (1994). As no lithogenic silica was present in algae cultures the second digestion step using HF was not necessary. Samples were digested in 20 ml of 0.2 mol l^{-1} Na(OH) for three hours at 95°C to ensure the dissolution of all $BSiO_2$ while keeping the solution far from solubility. After cooling, 5 ml of 1 mol l^{-1} HCl were added before centrifugation and analysis of the DSi concentration.

Dissolved silicon (DSi): DSi concentrations were determined in samples filtered using 0.2 µm syringe-filters according to the molybdate blue spectrophotometric method (Mullin & Riley 1965), adapted by Tréguer & Le Corre (1975) and modified by Gordon et al. (1993) to segmented flow colorimetry. An autoanalyser technicon type Bran and Luebbe was used.

Calculation of $BSiO_2$ dissolution rates: Different possibilities exist to calculate $BSiO_2$ dissolution rate from batch experiments. We used the linear initial rate approach described in the comparative study of Greenwood (2001). The initial specific $BSiO_2$ dissolution rate (R, in nmol Si s^{-1} g^{-1}_{BSiO2} or in % d^{-1}; Table 1 columns 5 and 6) was determined from the slope (R', nmol l^{-1} s^{-1}) of the first linear part of the DSi change versus time (initial dissolution rate) normalized to the initial concentration of $BSiO_2$ (w_i,

in g l^{-1} or in nmol l^{-1}; with 1 µmol = 60 µg $BSiO_2$). This initial phase (three to four first data points after the DSi concentration started to increase) lasted for five to ten days.

$$R = R' / w_i \qquad \text{eq. 2}$$

Normalization of dissolution rates to initial $BSiO_2$ concentration was necessary to compare experiments and treatments, as the increase of the DSi concentration depends on the initial amount of $BSiO_2$ (Kamatani & Riley 1979). It was impossible to start with exactly the same initial $BSiO_2$ concentrations in all treatments (Table 1 column 4). We therefore present specific $BSiO_2$ dissolution versus time to directly compare dissolution of aggregated and non-aggregated diatoms.

In addition to this initial specific dissolution rate, we calculated the daily specific dissolution based on the full duration of the DSi increase and will call this value the mean specific dissolution rate (Table 1 column 7). The amount of $BSiO_2$ dissolved after ten days (Table 1 column 8) was also calculated in treatments of sufficient duration.

Viability of cells: Viability of cells, e.g. the fraction of actively respiring cells was assessed in a companion study using the FDA (fluorescein diacetate) method (Garvey et al. in prep). The staining solution was prepared directly before use by dilution (1: 100) of the stock solution (50 mg of FDA in 10 ml of dimethylsulfoxide) with distilled water. After adding 100 µl of the FDA working solution to 9 ml of diluted (3 times) sample of the aggregate treatments or to 3 ml of undiluted sample of the freely suspended cells the ratio of living (fluorescing green cells) to total cell numbers was determined under a

Zeiss Axiovert-fluorescence microscope. At least 200 cells were counted at a magnification of 200 x in about 25 fields. Viability of cells was followed during the first 25 days of $BSiO_2$ dissolution of aggregated and non-aggregated *Thalassiosira weissflogii* (Garvey et al. in prep).

TEP: Three replicate samples (3 to 6 ml or 1 to 2 aggregates each) removed at the beginning and at the end of each dissolution experiment, were filtered onto 0.4µm polycarbonate filters and stained with 0.5 ml of a 0.02% aqueous solution of alcian blue in 0.06% acetic acid (pH 2.5) according to the method of Passow & Alldredge (1995b). After six milliliters of 80% H_2SO_4 were added, filters were soaked for two hours and the absorption measured at 787 nm in a 1 cm couvette. Each working solution of alcian blue was calibrated with Gum Xanthan. The units used are, Gum Xanthan equivalents per aggregate (µgXeq. agg^{-1}) for aggregate treatments, and Gum Xanthan equivalents per liter (µgXeq. l^{-1}) for freely suspended cell treatments.

Bacterial abundance: The method used to count bacteria in aggregates was based on one proposed by Velji & Albright (1993). Samples were diluted by a factor of 10 with 0.2-µm filtered artificial seawater and fixed with formaldehyde to a final concentration of 10 %. To break aggregates, tetrasodium pyrophosphat was added (final concentration of 0.5 to 10 mmol l^{-1}). Samples were vortexed several times during the 30-minute incubation time at room temperature and then sonicated for 60 s with a pistil sonicator (level 3-4) to break aggregates. Detailed initial tests showed that this procedure did not result in cell breakage, but destroyed aggregates efficiently. After gentle filtration,

bacteria were stained with 4',6-diamidino-2-phenylindole (DAPI) for a final concentration of 1 µg ml^{-1} (Velji & Albright 1993) in samples from both, the freely suspended cell and the aggregate treatments. Two replicate filters were prepared for each sample and the number of bacteria counted on each slide with a fluorescent microscope (Zeiss Axiovert). At least 300 bacteria per filter were counted in about ten fields at a magnification of 1000 x. The use of ultra-sonication necessary for the disaggregation of the bacteria prevented the enumeration of the fraction of bacteria attached to diatoms. Without ultra-sonication bacteria in aggregates were by far too dense to count.

Diatom counts: Samples were fixed with formaldehyde to a final concentration of 2 % and enumerated within one month. The use of a Coulter counter was impossible because of the presence of micro-aggregates and chains. Thus diatoms were enumerated using an inverted microscope (Zeiss) following the method of Utermöhl (1958). Utermöhl chambers were filled directly with about 3 ml of 10 to 20-fold diluted samples and cells were allowed to settle for two to three hours before enumeration. At least 300 cells were counted per sample at a magnification of 63 to 250 x.

Determination of DSi concentration in the pore water of aggregates: Concentrations of DSi in the pore water of aggregates were determined in five replicate aggregates at the end of treatment 7a with *S. costatum* and in 10 replicate aggregates of *C. decipiens*. The volume (V_a) of each individual aggregate was determined from its width (W_a) and length (L_a) measured under a binocular microscope (magnification 10 to 25 x). Each aggregate was transferred into a 50 ml polycarbonate tube filled with 40 ml

of artificial seawater (to a total volume of 50 ml). Then the artificial seawater surrounding the aggregate was carefully sampled and filtered to determine its DSi concentration (DSi_{ssw}; µmol l^{-1}). The tube was subsequently shaken to break the aggregate and mix the pore water of the aggregate with the surrounding artificial seawater. This solution was sampled again, filtered through 0.4 µm filters and the DSi concentration measured (DSi_f; µmol l^{-1}). Using this method, the elevated DSi concentrations in the boundary layer of the aggregates will be sampled together with the surrounding sea water and measurements of pore water DSi concentration corrected for elevated DSi concentrations in the boundary layer of the aggregates.

The number of diatoms (n) was also counted and the average cell volume (V_c; mm^3) calculated from the average cell width (W_c; mm) and length (L_c; mm) as determined by measuring 10 diatoms (eq. 3). The porosity (p) was calculated from the cell numbers in each aggregate, and from the volume of the cells (V_c; mm^3) and aggregates (V_a; mm^3) using equation 3 and 4. This calculation assumes the volume of TEP in aggregates to be negligible. The concentration of silicic acid inside the aggregate (DSi_i; µmol l^{-1}) was then calculated from equation 5. The calculated silicic acid concentrations in pore water are conservative estimates, as the presence of TEP decreases the value of the true porosity compared to the assumed porosity.

$$V_{a/c} = \pi / 6 * W^2_{a/c} * L_{a/c} \qquad \text{eq. 3}$$

$$p = (V_a - n * V_c) / V_a \qquad \text{eq. 4}$$

$$DSi_i = (DSi_f - DSi_{ssw}) * 0{,}05 / (V_a * p) \qquad \text{eq. 5}$$

II. RESULTS

$BSiO_2$ dissolution rates

Intraspecific comparison of the specific $BSiO_2$ dissolution rates of aggregated versus non aggregated cells

Because of species-specific differences in dissolution rates of diatoms, the specific dissolution rates of aggregated cells were compared to those of non-aggregated cells of the same species. Results of one representative experiment per species (of the 2-3 conducted for each) are presented in figures 2 a, b and c, which shows the percent of $BSiO_2$ that, dissolved over time and regressions that represent the initial specific dissolution rates (d^{-1}). As the dissolution rate may also vary with the physiological health of cells only treatments stemming from the same experiment are compared in each figure.

Thalassiosira weissflogii: Two strains of *T. weissflogii,* differing in their aggregation behavior and production of TEP were used. Strain 1 generated high TEP concentrations (15000 µgXeq. l^{-1}), but did not aggregate well. After one month, all cells had aggregated, including cells kept on the shaker table. Two experiments with 2 treatments each (1a, 1b, 2a, 2b; Table 1) were conducted with these aggregates formed from senescent cells, but no freely suspended cell treatments were possible. Cells from Strain 2 aggregated relatively quickly, within two to three days on the roller table, and remained non-aggregated on the shaking table. TEP concentrations from this strain were lower, around 7700 µgXeq. l^{-1}. These cells were used for experiment 3.

The initial specific dissolution rate of $BSiO_2$ in aggregates of experiment 3 was 5.4 % d^{-1} (10.4 nmol s^{-1} g^{-1}_{BSiO2}), while the respective value of freely suspended cells was

twice that (10.4 % d^{-1}; 20.0 nmol s^{-1} g^{-1}_{BSiO2}) (Fig. 2a). After ten days only 36 % of the initial $BSiO_2$ had dissolved if cells were aggregated, whereas 66 % of the initial $BSiO_2$ had dissolved from freely suspended cells. After 21 days the dissolution rate of aggregated cells had increased so that the dissolution curves intersect (Fig. 2a). At the end of the experiment, after 32 days 84 % of the initial $BSiO_2$ was dissolved in treatments with aggregates, whereas only 70 % of the initial $BSiO_2$ was dissolved in the treatment with freely suspended cells.

In experiments 1 and 2 DSi concentrations decreased during the first 24 to 48 hours, suggesting growth of *T. weissflogii*. Light exposure during transferal of aggregates and handling during preparations for the experiment may have sufficed to initiate this growth surge. Time zero for the calculations of $BSiO_2$ dissolution rates was set to day 2, when growth had ceased. The initial specific $BSiO_2$ dissolution rates of the four aggregated treatments with Strain 1 were appreciably lower than that of experiment 3 and averaged 3.0 ± 0.9 % d^{-1} (5.7 ± 1.7 nmol s^{-1} g^{-1}_{BSiO2}).

Chaetoceros decipiens: The initial specific $BSiO_2$ dissolution rates of aggregated *C. decipiens* (treatments 4a and b) were also lower than those of the freely suspended cells (treatment 4c) of the same experiment (Fig. 2b). About 40% of *C. decipiens* used in experiment 4 were initially viable and thus differed physiologically from those of experiment 5, where initially 60 % of cells were viable. As in experiment 4, initial specific $BSiO_2$ dissolution rates of aggregated *C. decipiens* of experiment 5 were lower than those of freely suspended cells. Thus, on average, the initial specific dissolution rates of aggregated cells of *C. decipiens* (2.4 ± 0.4 % d^{-1} or 4.6 ± 0.9 nmol s^{-1} g^{-1}_{BSiO2})

was substantially lower (2.5 fold) than the respective rate of freely suspended cells (5.9 ± 0.7 % d^{-1} or 11.4 ± 1.4 nmol s^{-1} g^{-1}_{BSiO2}; Table 1). During the first ten days of dissolution an average of 12 % of the initial $BSiO_2$ was dissolved in treatments with aggregated *C. decipiens*, whereas on average 34 % of the initial $BSiO_2$ was dissolved in the respective treatments with freely suspended cells.

Skeletonema costatum: $BSiO_2$ dissolution was determined in five treatments with *S. costatum* aggregates (6a, 6b, 7a, 7b and 8a) and two (6c, 8b) treatments with freely suspended *S. costatum* (Table 1). Experiments 6 and 7 were conducted with batches that aggregated within one to two days after transferal to the roller table, whereas the batch used for experiment 8 needed ten days to form aggregates.

On average the initial specific $BSiO_2$ dissolution rates of all four treatments with *S. costatum* aggregates was 2.6 ± 0.5 % d^{-1} (5.1 ± 2.6 nmol s^{-1} g^{-1}_{BSiO2}), or 1.9 ± 0.5 % d^{-1} (3.6 ± 1.0 nmol s^{-1} g^{-1}_{BSiO2}) if treatment 7a, where the initial dissolution rate was very high compared to that of the other treatments, is excluded. The respective value for the non-aggregated treatments was appreciably higher than both values with an average of 5.4 ± 2.9 % d^{-1} (10.5 ± 5.6 nmol s^{-1} g^{-1}_{BSiO2}; Table 1). Accordingly an average of 24 % of the initial $BSiO_2$ concentration dissolved during the first 10 days in aggregated treatments of *S. costatum*, whereas 36 % of the $BSiO_2$ dissolved in the respective treatments with freely suspended cells during the same time period. In experiment 8 (Fig. 2c), which lasted 21 days, the relative dissolution of aggregated cells surpassed that of freely suspended cells after 15 days, with a dissolution of 63 % and 53 % of the initial $BSiO_2$ for aggregated and non-aggregated cells, respectively.

Variability due to interspecific and physiological differences in specific $BSiO_2$ dissolution rates: Data from all experiments are shown in figure 3. Initial specific $BSiO_2$ dissolution rates of aggregate treatments (13 treatments) were all similar and below 5 % d^{-1} except in experiments 3a (5.4 % d^{-1}) and 7a (5.6 % d^{-1},), whereas those of freely suspended cells were larger than 5 % d^{-1} in all experiments except in experiment 8b where an initial specific rate of 3.4 % d^{-1} was determined (Fig. 3a). The Mann-Whitney W-test was used to statistically verify if the specific $BSiO_2$ dissolution rates of aggregated and freely suspended cells differed significantly independent of species-specific or physiological differences. All treatments and experiments were included in the statistical analysis. The median of the initial specific $BSiO_2$ dissolution rate of the 13 aggregate treatments was 2.5 % d^{-1}, whereas that of the five treatments with freely suspended cells was 6.4 % d^{-1}, resulting in a probability of 0.0058 (W = 61.0) that the initial specific dissolution rates of both treatment types were equal. This means that the initial specific dissolution rates of aggregated cells was statistically significantly lower compared to that of freely suspended cells despite the high variability between species, the impact of physiological stage and age and despite the large heterogeneity between replicate aggregates. A difference in the fraction of $BSiO_2$ dissolved after 10 days was also obvious between treatment types although here too variability was high within each type of treatment (Fig. 3b). The difference in the $BSiO_2$ dissolution rate depending on the aggregation state was less pronounced and not significant at 95 %, when the specific dissolution rate was calculated over longer time periods (over 14 days) because after 14 days $BSiO_2$ dissolution of aggregated cells surpassed that of freely suspended cells.

Changes in the specific $BSiO_2$ dissolution rates over time:

Specific dissolution rates depended on the time interval for which they were calculated. A comparison of column 6, 7 and 8 of the table 1 for each experiment shows that specific dissolution rates decreased with time except for the aggregated cell treatment 8a. In contrast to the other experiments specific dissolution rates in treatment 8a increased considerably with time (doubling from initially 1.8 d^{-1} to a mean rate of 3.6 d^{-1} over 18 days). In experiments 3 and 8 that are the only two experiments where the aggregated treatments were followed for more than two weeks, specific dissolution rates of aggregated cells decreased less with time than those of freely suspended cells. After two weeks specific dissolution rates of aggregated cells surpassed those of freely suspended cells.

DSi concentration in the pore water of aggregates

DSi concentration in the pore water of five aggregates, sampled at the end of treatment 7b, ranged from 9 to 180 µmol l^{-1}, whereas the DSi in the surrounding water was 6 µmol l^{-1} at the beginning and 13 µmol l^{-1} at the end of the experiment. The average porosity of these aggregates was about 99.9 %, ignoring the presence of TEP. TEP is thought to occupy about 5 % of the aggregate volume (Passow 2002a), and its impact on effective porosity is unclear. The DSi pore water concentration of 10 *Chaetoceros decipiens* aggregates, which were 48 h old, ranged from 80 to 230 µmol l^{-1}, with a surrounding seawater concentration below 20 µmol l^{-1}. The porosity of these aggregates was 99.8 %, excluding TEP.

Transparent Exopolymer Particles (TEP)

The concentration of TEP within aggregates varied by more than one order of magnitude, between 0.6 to 15 µgXeq. agg^{-1}, without consistent difference due to species. TEP concentration in the freely dispersed cell treatments also showed no consistent differences between species and averaged around 10 mgXeq. l^{-1}. Whereas no correlation could be found between $BSiO_2$ dissolution and TEP concentration in treatments with freely suspended cells, the initial specific $BSiO_2$ dissolution rate of aggregated cells was a function of TEP concentration in aggregates, independent of the species making the aggregate (Fig. 4). A higher TEP content reduced the initial specific $BSiO_2$ dissolution rate.

Bacteria

The concentration of bacteria in experiments 6 and 7 in aggregated and freely suspended cell treatments fluctuated over time without trend, but was very high ranging from 1.2×10^{10} to 1.6×10^{11} bacteria $aggregate^{-1}$ and 1.2 to 2.0×10^{10} bacteria ml^{-1}, respectively. Bacterial abundances in aggregates are difficult to compare directly to those of free living forms as the fraction of total bacteria that is associated with aggregates depends heavily on aggregate concentration. As we were investigating the impact of bacteria on $BSiO_2$ dissolution, the bacterial abundance in relation to diatom concentration is important. The number of bacteria normalized to the number of diatoms was similar or lower in aggregate treatments with on average 9×10^4 bacteria per diatom compared to treatments with freely suspended cells with on average about 26×10^4 bacteria per

diatom. However, the fraction of bacteria in close contact with diatoms (e.g. attached bacteria) is unknown. As it was necessary to disaggregate samples, attached bacteria could not be counted. Generally bacteria are not found on living diatoms, but colonize dead diatoms rapidly.

Viability

In a companion study, the change in viability of aggregated and dispersed *Thalassiosira weissflogii* over time was followed during 25 days (Garvey et al. in prep.) of a dissolution experiment (Fig. 5a, redrawn from Garvey et al. in prep.). The experiment started with a cell viability of 40 % for both aggregated and freely suspended cells. Viability of freely suspended cells decreased continuously until a viability of only 5 % was reached after 17 – 21 days. The viability of aggregated cells, in contrast, remained above 30 % for the first 13 days. Only thereafter did viability of aggregated cells decrease, reaching 7 % after about 25 days (Garvey et al. in prep.).

Independent of treatment, the fraction of $BSiO_2$ lost due to dissolution in both aggregated and freely suspended cell treatments was a function of the fraction of viable cells (Fig. 5b). The fraction of $BSiO_2$ lost increased from 40 % to near 80 % as the fraction of actively respiring cells (viability) decreased from 40 % to 5 %.

III. DISCUSSION

Applicability of laboratory determined specific $BSiO_2$ dissolution rates to the ocean

The size, weight, porosity, and TEP content of the aggregates made in roller tanks

for these experiments were similar to those of natural aggregates. Alldredge et al. (1998) observed a large size range of natural aggregates, and measured sizes ranged from 0.005 to 1 cm^3 per aggregate and weights from 5 to 1000 µg per aggregate. In our study aggregates were less variable with a size range of 0.1 to 1 cm^3 per aggregate and a weight between 187 and 622 µg per aggregate. Porosity of natural aggregates measured by Logan & Alldredge (1989) were slightly higher (between 99.94 and 99.98 %) compared to our aggregates made on roller tables (99.80 to 99.92 %). Natural aggregates contain between 0.5 and 100 µgXeq. agg^{-1} (Alldredge et al. 1998) and TEP concentrations of our aggregates ranged between 0.6 and 10 µgXeq. agg^{-1}. Bacterial numbers ranged from 8.1 x 10^9 to 1.6 x 10^{11} bacteria $aggregate^{-1}$, which is higher than the range observed on natural marine snow (10^6 to 10^8 bacteria $aggregate^{-1}$) (Silver & Alldredge 1981; Alldredge & Youngbluth 1985; Alldredge et al. 1986). Concentrations of TEP and bacteria in freely suspended cell treatments were within the upper range of naturally occurring concentrations (Passow 2002a) and typical for non growing dense batch cultures (Passow 2002b). Concentrations of aggregates and freely suspended cells in experiments had to be higher than commonly found in the ocean to be able to measure $BSiO_2$ dissolution reliably.

In our experiments, changes in the concentrations of DSi over time in treatments with aggregates potentially resulted from a combination of factors: (1) DSi uptake by diatoms, (2) release of DSi from diatoms, (3) $BSiO_2$ dissolution within aggregates and (4) exchange of DSi between aggregates and the surrounding seawater. Uptake and release of DSi by cells is negligible after one to two days in the dark (Blank & Sullivan 1979). Therefore, changes in DSi concentrations over time in treatments with aggregates reflect

an apparent dissolution of $BSiO_2$, i.e. true dissolution combined with transport of DSi through aggregates (diffusion and advective flow). *In situ*, dissolution and transport processes are also combined so that this "apparent dissolution" accurately reflects silica release into the water column, and represents the value necessary to budget silica recycling during sinking.

The average initial specific $BSiO_2$ dissolution rates measured for freely suspended cells (6.6 ± 2.6 % d^{-1} corresponding to 24.2 ± 9.5 yr^{-1}) and aggregates (2.9 ± 1.1 % d^{-1} corresponding to 10.0 ± 4.2 yr^{-1}) are in the same range as the estimated global world average of 16 yr^{-1} (Van Cappellen et al. 2002) and as *in situ* measurements compiled by Nelson et al. (1991) which reach from 5 to 1000 yr^{-1}.

Factors responsible for the difference in $BSiO_2$ dissolution rates between aggregated and freely suspended cells

In our study, initial specific $BSiO_2$ dissolution rates of aggregated cells were two to three times lower than those of non-aggregated cells. The lower initial dissolution rates can be explained by a combination of factors, which are first discussed individually and thereafter their interaction will be addressed.

DSi concentrations in pore water

Average DSi concentrations in aggregate pore water were 100 µmol l^{-1}, i.e. appreciably higher than DSi concentrations in the surrounding seawater, which averaged around 20 µmol l^{-1} for all experiments. Brzezinski et al. (1997) also found the DSi concentrations in pore water of natural aggregates to be elevated (305 µmol l^{-1}) compared

to the DSi concentration of the surrounding seawater.

With natural DSi concentrations remaining far below saturation (solubility of 1000 µmol l^{-1} to 1500 µmol l^{-1} at a temperature of 5°C to 15°C, respectively; Van Cappellen & Qiu 1997a, 1997b), it has long been believed that the rate of $BSiO_2$ dissolution in the water column would not be influenced by DSi concentrations. Van Cappellen & Qiu (1997a, 1997b), however, demonstrated a non linearity between dissolution and DSi concentration. Far from equilibrium, at concentrations smaller than 200 µmol l^{-1} a small increase in DSi has a large effect on the dissolution rate. Although their curve does not extend to lower concentrations, their results indicate a large change in the dissolution rate with relatively small changes in DSi concentration in the range of DSi concentrations found in the ocean and used in our experiments. The elevated DSi concentrations measured in the aggregate pore water (around 100 µmol l^{-1}) should therefore contribute to the slower dissolution of aggregated diatoms relative to non-aggregated diatoms.

Viability

Aggregation of cells has been suggested to represent an evolutionary adaptation for survival (Smetacek 1985, Gotschalk & Alldredge 1989). The viability of *T. weissflogii* is prolonged by aggregation (Garvey et al. in prep.). An extended viability of aggregated cells is possibly due to the elevated nutrient concentrations found within aggregates (Shanks & Trent 1980, Grossart & Simon 1993) as high nutrient concentrations decrease cell mortality. Prolonged viability directly affects $BSiO_2$ dissolution, because the frustules of living diatoms are protected against dissolution by an organic membrane (Patrick & Holding 1985, Bidle & Azam 1999). Consequently, $BSiO_2$ dissolution rates in

living cells are close to zero (Nelson et al. 1976). The dissolution of diatom frustules commences only after cells are senescent or dead, and the organic coating surrounding the diatom frustules is removed by bacteria or chemical cleaning (Kamatani 1982). In our experiment, viability of cells seemed to determine $BSiO_2$ dissolution independent of the treatment suggesting that the difference in viability was indeed responsible for the retardation of dissolution in aggregated cells (Fig. 5b). At the end of experiment 3 and 8 which lasted more than two weeks aggregated cells dissolved faster once aggregates aged. These observations suggest that aggregation of diatoms delays the dissolution of frustules rather than decreasing it. This is consistent with the impact of aggregation on diatom viability, which remains higher for longer within aggregates.

Bacteria

In nature, bacteria remove the protective organic layer after cells are senescent, and bacterial activity is a prerequisite for dissolution of diatom frustules (Patrick & Holding 1985, Bidle & Azam 1999, 2001). TEP form a good substrate for bacteria (Passow & Alldredge 1994) and bacteria associated with aggregates are very concentrated and active (Smith et al. 1992). This could imply that the removal of the protective organic layer should be faster in aggregates, which, all other things being equal would subsequently lead to faster dissolution of aggregated diatom frustules. We, however, found the opposite to be true; initial $BSiO_2$ dissolution was slower in aggregates.

Bacterial numbers normalized to diatom numbers were on average slightly higher in the freely suspended cell treatments than in the aggregate treatments. But bacteria need to be closely associated with senescent or dead cells to be able to degrade the protective

layer. We have no estimate of the fraction of bacteria directly attached to diatoms as preparations for counting bacteria disaggregated them. However, the relatively high ratio between bacteria and diatoms suggest that upon senescence diatoms could be populated rapidly by bacteria in both treatments. Thus bacterial concentration or activity was probably not a rate limiting step for $BSiO_2$ dissolution. Rather than bacterial abundance the lower fraction of senescent and dead diatoms in aggregates was presumably responsible for a lower degradation rate of the protective layer and thus the lower $BSiO_2$ dissolution rate. This is confirmed by the relationship between $BSiO_2$ dissolution and viability for aggregated and freely suspended cells. This relationship suggests that not aggregation *per se*, but increased viability of cells may be responsible for the reduced initial dissolution rate found in aggregated *versus* freely suspended cells.

Transparent Exopolymer Particles (TEP)

Initial specific $BSiO_2$ dissolution rates in aggregates were significantly correlated with the TEP content, even when aggregates consisting of different species were included (Fig. 4). The reduction of the initial $BSiO_2$ dissolution rate with increasing TEP content as well as elevated DSi concentrations in aggregate pore water, imply that TEP may play a role in decreasing DSi flux out of aggregates and therefore the initial $BSiO_2$ dissolution rate of aggregated cells. A reduction of the diffusion of solutes through mucus coated sediments compared to sediments in free solution has been attributed to both size and charge inhibition due to mucus (Hannides et al. 2005). Porous sediments covered with TEP-like mucus are to some extent comparable to marine snow-sized aggregates, although the porosity of aggregates is much higher. Possibly chemically binding of DSi

to TEP (Welch & Vandevivere 1994, Welch & Ullman 1993), charge restrictions, a lengthening of the diffusion pathway due to the fractal nature of diatom aggregates (Dachs & Bayona 1998), or size dependant permeability are responsible for the accumulation of DSi in aggregates. Alternatively, TEP may simply allow elevated DSi concentrations to build up by restricting advective flow, without impacting diffusion rates appreciably (Ploug & Passow subm.). In all cases TEP would contribute to the reduction of the apparent dissolution of $BSiO_2$ by retaining DSi within aggregates. Higher DSi concentrations within aggregates not only reduce dissolution rates directly, but higher nutrient concentrations in general are presumably also responsible for the higher survival rate of diatoms inside aggregates. The higher viability of aggregated diatoms in turn protects the cells against bacterial degradation of the organic membrane and the onset of $BSiO_2$ dissolution.

Chain or colony formation

It is interesting to note that within the freely suspended treatments the initial $BSiO_2$ dissolution rate of *T. weissflogii* was by far the highest compared to the other two species. Compared to *C. decipiens* and *S. costatum*, which both form chains, individual *T. weissflogii* cells are farther apart from each other. The shape, size and the degree of silicification of cells all impact dissolution rates. Our results indicate that the proximity to other cells, which is higher in chain forming species compared to non-chain forming species may also impact $BSiO_2$ dissolution rate. Elevated concentrations in the microenvironment (diffusive boundary layer) surrounding individual cells, may affect neighboring cells of chain forming species. Thus, chain formation, like aggregation may

be an adaptation to increase the proximity of cells to each other, which may enable the development of a micro-environment that promotes an increase in the overall viability of the population.

IV. CONCLUSION

$BSiO_2$ dissolution rates measured experimentally on laboratory-made aggregates and on freely suspended cells show evidence of a decreased specific $BSiO_2$ dissolution rate within aggregates during the first 14 days. This retardation of dissolution seems to occur because aggregates maintain a high DSi concentration (>100 µmol l^{-1}) within their pore water, possibly due to the presence of TEP, which in turn extends the viability of aggregated cells compared to freely suspended cells. The possible effect of the retardation of diffusion due to TEP will be investigated further with the help of a model (Moriceau et al. in revision). A higher viability will inhibit bacterial degradation of the protective organic membrane. Apparently, in our experiment, the degradation of the protective layer after cell death was fast enough to have had little impact on the difference between $BSiO_2$ dissolution rates measured in both treatments. Instead $BSiO_2$ dissolution rate was a function of the viability of cells.

Our results bring interesting elements in the debate concerning the ecological role of aggregation (Smetacek 1985; Logan & Alldredge 1989). In some areas of the ocean, e.g. areas with upwelling, as well as in certain circumstances when aggregates do not sink (Rinaldi et al. 1995) an increase in the survival time of cells by 10 to 20 days will appreciably increase the probability of the cells to again find themselves in growth favoring conditions. Aggregation appears less advantageous at least for the surface

ecosystem under different circumstances. According to our results and assuming a sinking velocity for aggregates of 100 m d^{-1} (Alldredge & Gotschalk 1988, Alldredge & Silver 1988) diatom aggregates would reach a depth of more than 1400 to 1700 m before dissolution rates of aggregated cells would increase to values similar to those of freely suspended cells. The DSi released at this depth then needs to be transported back to the euphotic zone before it can support renewed diatom growth. In this case, aggregation will not favor sustained diatom production in surface waters. Of course, these aggregate will play another ecological role for flux feeders inhabiting the mesopelagic layer (Jackson 1993) and one might suspect that the activity of such organisms will redistribute the depth of $BSiO_2$ dissolution over a wide range.

ACKNOWLEDGEMENTS:

We are grateful to everyone in the C-group of the AWI for their support during experiments. Thanks to A. Engel for her contributions in the initial stages of our research, to M. Ernst and S. Säuberlich for their help with bacteria enumeration, to A. Terbrüggen, M. Wolff, G. Langer, M. Briand and R. Marc for their technical assistance. Thanks to S. ni Longphuirt and Christina De La Rocha who kindly read earlier versions and to J. Grall for his help with statistics. This work has been funded by the EU, which is acknowledged, partly through the ORFOIS (EVK2-CT2001-00100) project and partly through the Si-WEBS (HPRN-CT-2002-00218) Research Training network of the Marie Curie program. This is contribution # xxx of the IUEM and xxx of the AWI.

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FIGURE LEGENDS:

Figure 1: Explanatory diagram of the set-up of the dissolution experiments. In the first step, a dense batch culture was grown, one aliquot of this culture was transferred into a four liter rolling tank (preparation for aggregate treatment), whereas a second aliquot was incubated in a 1 liter flask on a shaker table (preparation for freely suspended cell treatment). During step 2 aggregates collected from the roller tank were isolated and transferred into a smaller roller tank filled with artificial seawater without nutrients. Freely suspended cells were allowed to sediment and the overlaying media was carefully exchanged for nutrient-free artificial seawater. During the dissolution experiment itself, aggregates were once again incubated on a roller table and freely suspended cells kept dispersed and in suspension by mild shaking on a shaker table. During all phases both treatments were incubated at 13°C, and except the growth phase of the culture before step 1, in the dark.

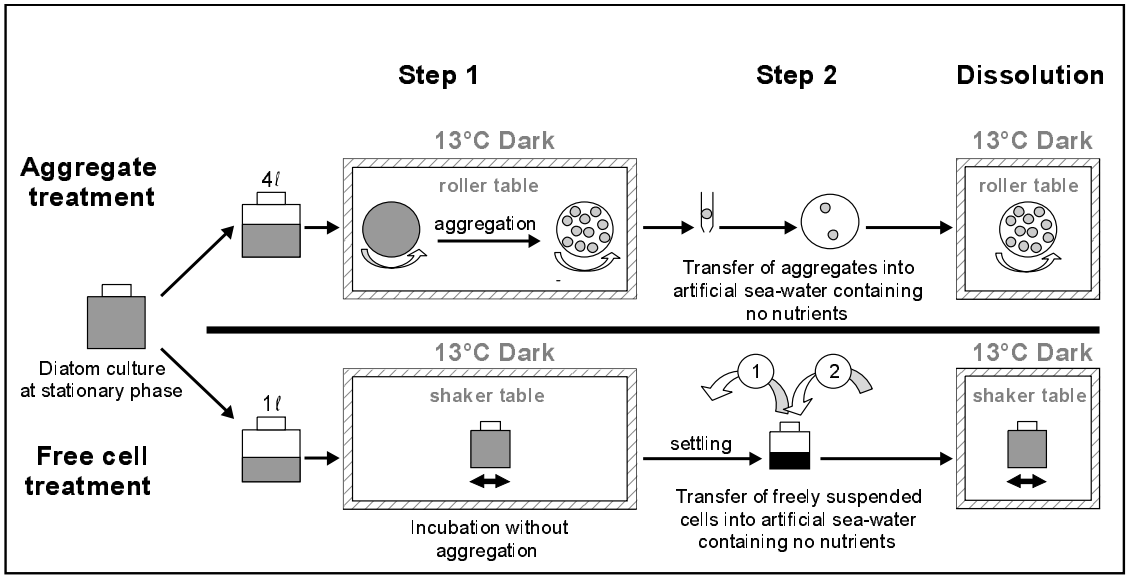
Figure 2: Initial specific dissolution rate of biogenic silica ($BSiO_2$) versus time expressed as the increase in the concentration of dissolved silica [DSi] relative to t = 0 (released DSi) normalized to the concentration of initial $BSiO_2$, in percent. a) Experiment 3a and b, with aggregated (empty diamonds) and freely suspended (full diamonds) *Thalassiosira weissflogii*. b) Experiment 4a, b, with *Chaetoceros decipiens* aggregates (empty diamonds and triangles) and experiment 4c, with freely suspended *C. decipiens* (full diamonds). c) Experiment 8a and b, respectively, with aggregated (empty diamonds) and freely suspended (full diamonds) *Skeletonema costatum*. Regression lines (least square

fits) are calculated for the initial time period when DSi increase was linear and are not forced through zero. Each graph represents data from one experiment only, which means that cells originated from the same batch and were physiologically speaking identical – except for the status of aggregation.

Figure 3: Comparison of initial specific dissolution rates (a) and specific dissolution after 10 days (b) for aggregated (n = 5, 3, 5) and freely suspended (n = 1, 2, 2) treatments of *Thalassiosira weissflogii, Chaetoceros decipiens* and *Skeletonema costatum,* respectively. More experiments with aggregated cells were conducted, as the variability between replicate aggregates was high. In (b) both data points of $BSiO_2$ dissolution of free cells of *Skeletonema costatum* lie on top of each other. Estimates of $BSiO_2$ dissolution using either calculation show a statistically significant (see text) lower specific dissolution rate when cells are aggregated, although variability due to physiological and species specific differences as well as due to variability of replicate aggregates is high.

Figure 4: Initial specific dissolution rate of $BSiO_2$ in aggregates (treatments 2a, 5a, 6a, 6b, 7a, 7b) of *S. costatum* (4 experiments), *C. decipiens* (1 experiment) and *T. weissflogii* (1 experiment) as a function of TEP concentration in aggregates (regression line, $r^2 = 0.78$, n = 6 experiments, $p < 0.05$).

FIGURE AND LEGENDS:



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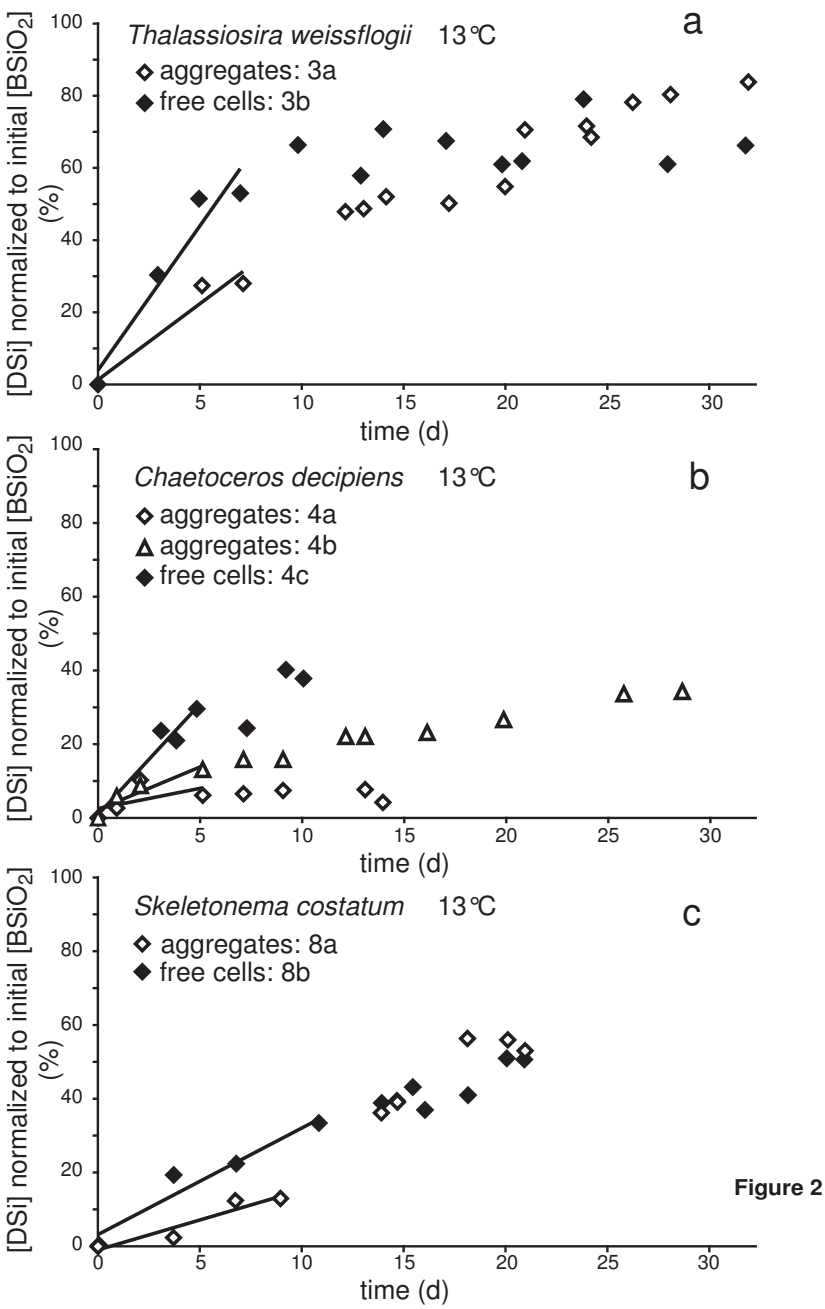

Figure 2: Initial specific dissolution rate of biogenic silica ($BSiO_2$) versus time expressed as the increase in the concentration of dissolved silica [DSi] relative to t = 0 (released DSi) normalized to the concentration of initial $BSiO_2$, in percent. a) Experiment 3a and b, with aggregated (empty diamonds) and freely suspended (full diamonds) *Thalassiosira weissflogii*. b) Experiment 4a, b, with *Chaetoceros decipiens* aggregates (empty diamonds and triangles) and experiment 4c, with freely suspended *C. decipiens* (full diamonds). c) Experiment 8a and b, respectively, with aggregated (empty diamonds) and freely suspended (full diamonds) *Skeletonema costatum*. Regression lines (least square fits) are calculated for the initial time period when DSi increase was linear and are not

forced through zero. Each graph represents data from one experiment only, which means that cells originated from the same batch and were physiologically speaking identical – except for the status of aggregation.

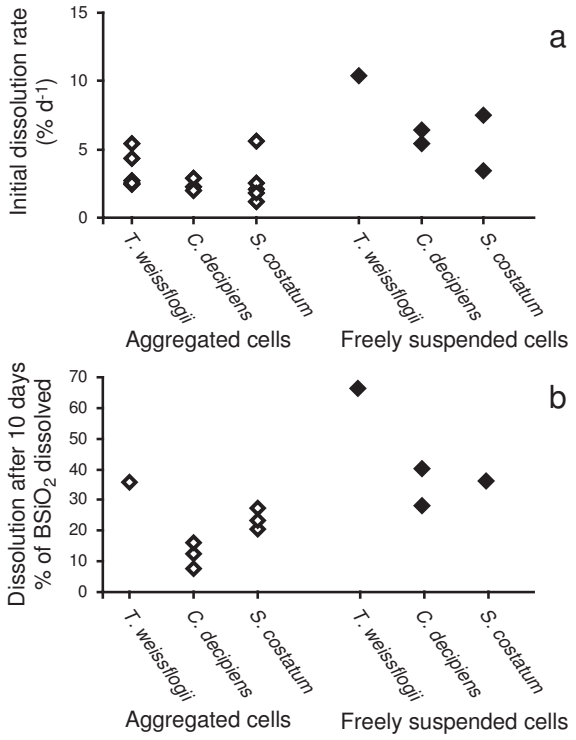

Figure 3

Figure 3: Comparison of initial specific dissolution rates (a) and specific dissolution after 10 days (b) for aggregated (n = 5, 3, 5) and freely suspended (n = 1, 2, 2) treatments of *Thalassiosira weissflogii, Chaetoceros decipiens* and *Skeletonema costatum,* respectively. More experiments with aggregated cells were conducted, as the variability between replicate aggregates was high. In (b) both data points of $BSiO_2$ dissolution of free cells of *Skeletonema costatum* lie on top of each other. Estimates of $BSiO_2$ dissolution using either calculation show a statistically significant (see text) lower specific dissolution rate when cells are aggregated, although variability due to physiological and species specific

differences as well as due to variability of replicate aggregates is high.

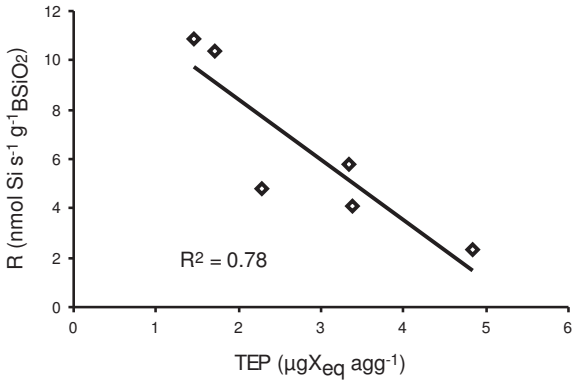

Figure 4

Figure 4: Initial specific dissolution rate of $BSiO_2$ in aggregates (treatments 2a, 5a, 6a, 6b, 7a, 7b) of *S. costatum* (4 experiments), *C. decipiens* (1 experiment) and *T. weissflogii* (1 experiment) as a function of TEP concentration in aggregates (regression line, $r^2 = 0.78$, n = 6 experiments, $p < 0.05$).

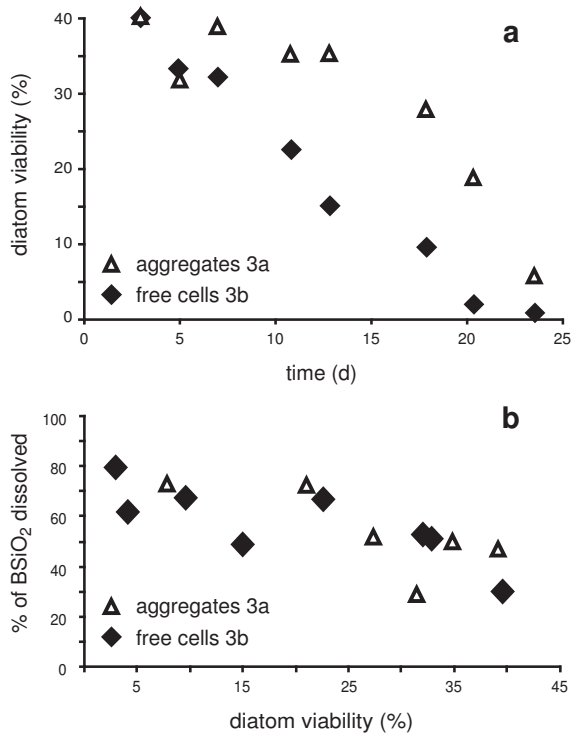

Figure 5

Figure 5: (a) Decrease in viability of diatoms in aggregates (empty triangles) and freely suspended cells (full diamonds) versus time during a dissolution experiment with *T. weissflogii*. Redrawn from Garvey et al. in prep (b) Fraction of $BSiO_2$ dissolved (*T. weissflogii*) as a function of viability with data of both, aggregated and freely suspended treatments included.