METHODS

2.1 General set up. The experiment consisted of two treatments (Present and Future) representing different pCO_2 conditions and two sequential experimental phases: the cell growth phase and the aggregation phase. The experiment was sequentially replicated. The two replicates are going to be referred as Experiment 1 and Experiment 2.

2.2 Cell growth phase. During the cell growth phase, the effect of OA was tested on a culture of *S. marinoi* that was incubated for five days, in 5 l transparent bags. Two replicate bags were used for each treatment. The pH was measured daily immediately after collection in each bag, while the TA samples were collected for later analysis in one sample per treatment at the beginning and at the end of the cell growth phase. The algal cells were counted, sized and their instantaneous *in vivo* chlorophyll fluorescence (Ft) and quantum yield (Qy) were measured daily in the morning. The concentration of bacteria and TEP were measured at t_0 ,$t_{2.8}$ and $t_{4.6}$. The nutrients concentration was sampled at $t_{2.8}$ and $t_{4.6}$. The dry weight, particulate carbon, hydrogen and nitrogen were measured at $t_{4.6}$. After 5 days the cultures in the two bags per treatment were pooled, the carbonate system was readjusted to the starting conditions and subsequently they were inoculated into cylindrical rolling tanks.

2.3 Aggregation phase. The aggregation phase consisted in the incubation of the cultures into cylindrical tanks on rolling tables (Edmondson, 1989). A total of 8 tanks (4 replicates per treatment) were incubated over the rolling table for two days in the darkness in the environmental room at 15°C. Solid body rotation is established in these rolling tanks within less than three hours (Ploug, Terbrüggen, Kaufmann, Wolf-Gladrow, & Passow, 2010) and the sinking of particles through the water column was simulated and aggregation promoted. After 38 hours of incubation the aggregates sinking velocity, number and size were measured. The algal cell number, bacterial cell number, TEP, dry weight, particulate carbon, hydrogen and nitrogen were evaluated, both for the aggregated fraction and for the surrounding water. The carbonate system was characterized by measuring pH in all the tanks and TA in one tank per treatment.

2.4 Carbonate system manipulation and characterization. The carbonate system was manipulated chemically using HCl, NaOH, $NaHCO_3$ and Na_2CO_3 in a closed system without headspace (Rost, Zondervan, & Wolf-Gladrow, 2008). The artificial seawater (ASW) was prepared to contain a 2050 µmol/kg DIC by adding $NaHCO_3$.

The pH of the ASW was then measured and adjusted to ~ 8.0 by addition of NaOH for the present treatment. At the ambient temperature of 15 ºC the pCO2 of this treatment was thus 390 µatm, as calculated using CO2sys

(Lewis, Wallace, & Allison, 1998). The additions of HCl, $NaHCO_3$ and Na_2CO_3, necessary to reach a target pCO_2 of ~1000 µatm for the future treatment, required a pH of ~ 7.7 at 15 ºC, calculated using CO2sys. During the experiment the carbonate system was fully characterized by measuring pH and total alkalinity (TA) and calculating the other parameters by using the software CO2sys. The dissociation constants K1 and K2 from(R. Roy, Roy, Vogel, & Porter-Moore, 1993) and $KHSO_4$ according to (Dickson, 1990) were used. Carbonate parameters were calculated for our experimental temperature (15°C) and salinity (34.2 ppm). The pH (total scale) was measured colorimetrically with the spectrophotometer 10S Vis (Genesys) using the dye m-cresol purple (Clayton & Byrne, 1993) following the procedure described by Dickson in the SOP 6b (Dickson, Sabine, & Christian, 2007). TA was determined using acidimetric open cell titration as described in the SOP 3b and expressed as µmol/Kg SW(Dickson et al., 2007). The titrator used was a 765 Dosimat (Metrohom) owned by the Romberg Tiburon Center for Environmental Studies (SFSU). The accuracy of the instrument was checked at the beginning of each run using Certified Reference Material (CRMs) from A.G. Dickson (SIO, Oceanic Carbon Dioxide Quality Control). Samples for TA determination were stored in glass bottles with no headspace and fixed with mercuric chloride until analysis.

2.5 Algal culturing. The organism used in this study was the strain CCMP 1332 of the diatom *S. marinoi* isolated by the Pavasoli – Guillard National Center for Marine Algae and Microbiota. The Strain was collected in 1956 in Milford, Connecticut, USA (41.2264N 73.0639W). The culture was grown into artificial seawater (ASW) according to (Kester, Duedall, Connors, & Pytkowicz, 1967).

Closed batch cultures were grown for 5 days in 5L resin polymer and polyethylene mix bags (Scholle packaging). The algal cultures were grown under fluorescent bulbs at 60 µE/m^2/sec with a 12h:12h photoperiod inside the environmental room kept at 15°C. The light was adjusted using a light meter LI-185B (LICOR) with a flat sensor. The nutrients concentrations of the media were the following: NO_3^- 70.7 µM, PO_4^{3-} 4.4 µM, $Si(OH)_4$ 64.2 µM, vitamins and metals as in f/2 (Guillard & Ryther, 1962). The bags were inoculated to an initial cell concentration of about 20000-25000 cells/l. The culture used in the experiment was

not axenic but the number of bacteria was severely reduced a few days before the experiments by sieving the culture five times through a 10 µm plankton sieve and washing it with sterile ASW. During the experiment, the algal concentration was checked using a Neubauer counting chamber under an optical microscope (Olympus CX41). Length (pervalvar axis) and width (diameter) were recorded for 20 different cells per sample chosen randomly from different chains. The Ft and the Qy were measured using the Z985 cuvette Aqua Pen (Qubit System Inc.).

2.6 Bacteria. The concentration of bacteria was determined by counting 4',6-diamidino-2-phenylindole (DAPI) stained samples using an epifluorescence microscope (Olympus BX51) equipped with a digital camera operated with the software QCapture Pro (QImaging). Samples were fixed with formalin and kept in the fridge until their filtration and staining. The cells concentration was estimated by counting cells over the entire ocular field in 10 random replicates per sample.

2.7 TEP. TEP concentration was measured colorimetrically on 4 replicates per sample by filtration on 0.4 µm PC filters (Poretics) and staining with Alcian Blue using the semi-quantitative method described in (Passow, 1995). The volume filtered was chosen accordingly to the cell concentration.

2.8 Nutrients. Phosphate, and Nitrate + Nitrite were measured in the Marine Science Institute Analytical Lab, with a continuous flow technique for automated wet chemical analysis, using the flow injector analyzer Quik Chem 8000 (Lachat Instruments).

2.8 Particulate carbon, hydrogen and nitrogen (CHN). Prior to sampling, GF/F filters (25 mm diameter) were combusted at 450°C and weighted with the digital balance for dry weight and POC/PON analysis. 150 ml samples duplicates were filtered at low pressure onto the GF/F filters, rinsed with milli-Q water and then dried overnight at 60°C in a drying oven. Once dried, the filters were reweighted and then POC and PON were determined by high temperature combustion (Dumas method) with the organic elemental analyzer CEC 440HA (Control Equipment Corporation) in the MSI analytical lab.

2.9 Rolling tanks. The tanks had a volume between 1.1 l and 1.2 l and were made of acrylic. The volume of culture necessary in order to obtain a cell concentration of 120000 cells/ml was inoculated into each tank, which was then topped off with ASW without nutrients and with the carbonate chemistry adjusted for the respective treatment. The rotation speed was set at 1 RPM, which allowed the sinking of

aggregates without their collision with the container walls. After 38 hours of incubation the tanks were removed from the rolling table, gently placed horizontally over a transparent glass and photographed from below using the digital camera G12 (Canon) mounted on a tripod.

2.10 Aggregate characterization. Aggregate sizes and abundance were determined in each tank by image analysis of photos taken when aggregates settled to the bottom of the tank. A ruler was included in the picture next to the tank for reference. The pictures were processed with Photoshop CS4 Extended (Adobe). The aggregates were isolated from the background of the image with an automated color selection, counted and their total area was measured. The mean equivalent spherical diameter of the aggregates was also calculated. The sinking velocity of all aggregates >1 mm was measured in cylindrical sedimentation column. The column was filled with 1 l of the ASW from the respective treatment and allowed to stabilize fro 30 min in the environmental room at 15 °C. Aggregates were gently introduced into the column, one at a time, by allowing them to sink out of the disposable 10-ml plastic pipette. The pipette was expressly modified, by cutting off its tapered end. The descent was followed using a stopwatch over a distance of 24.8 cm. Aggregates that fragmented during handling were not measured. The sinking velocity was expressed in m/day. When sinking velocity of all aggregates of each tank were measured, the column containing all aggregates was mixed well to create homogeneous aggregate slurry.

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