METHODS

Formation of TEP and aggregates by the diatom *Thalassiosira weissflogii* (CCMP 1336) was investigated as a function of temperature, pCO2 and the presence or absence of the bacteria *Marinobacter adhaerens* HP15 (Kaeppel et al. 2012, Gaerdes et al. 2010), using a full factorial design. Three different carbonate chemistry regimes were selected to reflect: (i) present-day conditions, with the partial pressure of CO_2 (pCO_2) ranging between 300-350 µatm (termed ambient) and two future ocean scenarios with pCO_2 ranging from 750-850 µatm (designated future 1) and 1000-1250 µatm (referred to as future 2). Two temperatures were chosen, 15 ℃ and 20 ℃, and each combination of environmental conditions was tested with an axenic diatom culture and with diatoms plus *M. adhaerens*. Prior to the aggregation experiment, the diatom and bacterial cultures were acclimatized to the respective temperature and carbonate chemistry regimes for more than 8 generations. During this acclimatization phase diatoms were kept in the exponential growth by regular dilutions. The diatom was grown in artificial seawater (Kester et al. 1967), the bacteria in marine broth prepared with ASW. After the acclimatization, aggregation experiments were conducted in duplicates in roller tanks in darkness. Replicate roller tanks were set-up with diatom cells at a final concentration of 3 x 10^3 cells ml^{-1} and – where appropriate - bacteria at a final concentration of 3 x 10^5 cells ml^{-1}.

Total alkalinity (TA), pH and DIC were monitored regularly during both the acclimatization and aggregation phases. TEP concentrations were determined at the beginning of the aggregation phase. The aggregation phase was terminated after 96 hrs on the rolling table, and the number and size of aggregates in each tank measured. The aggregates were removed manually, their sinking velocities determined and all aggregates of each tank pooled to form aggregate slurry. TEP concentrations were measured in the aggregate slurry and the surrounding (aggregate free) seawater (SSW).

The carbonate chemistry was perturbed by manipulating pH and dissolved inorganic carbon (DIC) concentrations chemically; e.g. by adding appropriate amounts of 0.1 M

HCl (mL kg^{-1}), 0.1 M $NaHCO_3$ (mL kg^{-1}) and 0.001 M Na_2CO_3 (mL kg^{-1}). The carbonate system of the experiments was monitored by measuring pH, TA and DIC. The pH (total scale) was measured with a spectrophotometer using the indicator dye m-cresol purple (Sigma-Aldrich) within 1-2 hours of sampling at 25 ºC (Clayton and Byrne 1993). Samples for TA and DIC measurements were collected following SOP1 (Dickson and Goyet 1994). TA and DIC samples were poisoned with 0.02% saturated $HgCl_2$ by volume and analyzed at the Dickson Laboratory at the Scripps Institution of Oceanography, UCSD. The carbonate chemistry was calculated using CO_2Sys (Lewis and Wallace 1998).

Diatom cell abundance was monitored daily by counting cells in a Sedgwick-Rafter Cell S50 (SPI Supplies, West Chester, PA, USA) using an inverted Axiovert 200 microscope (Zeiss, Jena, Germany). The axenicity of the diatom culture was intermittently checked by epifluorescence microscopy after staining with the dye 4′, 6-diamidino-2-phenylindol (DAPI) (Porter and Feig 1980).

The sinking velocity of 10 aggregates per tank was determined by measuring the time needed for each aggregate to sink 20 cm. The dimensions of the aggregate axes (x, y, and z direction) were measured under a dissecting microscope, and the aggregated volume calculated assuming an ellipsoid shape. The equivalent spherical diameter (ESD) was calculated. The aggregates were classified into two size classes: large aggregates of size ≥ 5 mm ESD and smaller aggregates of size < 5mm ESD.

TEP concentrations were measured colorimetrically as described by Passow and Alldredge (1995). The staining solution was calibrated using Gum Xanthan, and TEP was expressed as Gum Xanthan equivalents per liter (GXeq L^{-1}). TEP was measured in four replicates per tank.

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