Respective publication:
Passow, Uta, Christina L. De La Rocha, Caitlin Fairfield, Katrin Schmidt submitted to L&O in Dec 2012 : Aggregation as a function of pCO_2 and mineral particles

Methods

Roller tanks experiments: 14 cylindrical five-liter roller tanks/ treatments (see table) rotating at 1 rpm encompassing three acidification scenarios and 5 concentrations of the clay illite incubated at 14ºC in continuous darkness for 44 to 48 hours. Solid body rotation was established within three hours.

Each tank was filled with a phytoplankton-detritus-seawater mixture prepared in a large (150-liter) container. This mixture was produced by combining (i) 7 twenty-liter containers of ‘bottle blooms” (1-2 µg Chl. a L^{-1}) grown for 2-4 days in nutrient-enriched surface water collected from the Santa Barbara Channel (34° 23.134'N 119° 50.823'W) and (ii) 4 one-liter cultures each of *Thalassiosira weissflogii* (60000 cells mL^{-1}) and *Chaetoceros affinis* (40000 cells mL^{-1}) grown in F/20 media, and (iii) 70 mL of concentrated fecal pellet detritus from sea urchins (that had been frozen to kill any eggs). This initial phytoplankton-detritus-seawater mixture had a pH_T of 7.75 (total scale), a salinity of 33.2‰, a total alkalinity (TA) of 2295 mol kg^{-1}, 64 µM silicic acid and 4.4 µM phosphate. This mixture was used in all experimental treatments.

The carbonate system was left unaltered in tanks 1-5 (ambient), adjusted to ambient + 260 ppm pCO_2 for tanks 6-9, and adjusted to ambient + 520 ppm CO_2 in tanks 10-14. Different volumes of a concentrated suspension of clay (illite) were added to all tanks but 1, 6 and 10, yielding an array of clay concentrations of 0 to 10 mg/L (table). Each treatment was sampled at the beginning and end of the experiment.

At the end of the experiment, each tank was individually removed from the roller table and placed on its side. Samples for the determination of the carbonate system were collected from the aggregate-free surrounding seawater (SSW) after the aggregates had settled to the bottom of the cylindrical tank. The settled aggregates were then photographed for image analysis of their size and total number. Following this, aggregates $\geq$ 1 mm were isolated manually using a 10-ml disposable pipette whose narrow tip had been cut off. The first ten aggregates were sized and their sinking velocity measured. All of the aggregates were mixed together and this aggregate slurry was subsampled for dry weight (DW), particulate organic and inorganic matter (POM and PIM), particulate organic carbon and nitrogen (POC and PON), transparent exopolymer particles (TEP), and dissolved organic carbon (DOC). Aggregates in this paper are thus defined as particles > 1 mm. Samples for the determinations of DW, PIM, POM, POC, PON, TEP, and DOC were also collected from the SSW that remained in the tanks after aggregates were removed. The primary particles and aggregates < 1 mm in the remaining SSW then caught on GF/F filters will be referred to here as particulate matter. Material passing the filters is considered dissolved.

Carbonate System:

We perturbed the carbonate system using the closed system approach. No perturbations were used for treatments reflecting ambient conditions. Future conditions were generated by adding appropriate amounts of 0.1 mol L^{-1} HCl, 0.001 mol L^{-1} Na_2CO_3, and 0.1 mol L^{-1} $NaHCO_3$ in a closed system to result in the high CO_2 scenarios, labeled future 1 (ambient + 260 ppm pCO_2) and future 2 (ambient + 520 ppm pCO_2), without bubbling the water. The respective additions needed for the perturbations as well as the carbonate chemistry during the experiment were calculated using CO2Sys . The dissociation constants K_1 and K_2 from Mehrbach et al, 1973 refit by Dickson and Millero, 1987 and K_{HSO4} according to Dickson were used. The carbonate system was monitored both at the beginning and end of the experiment by measuring pH (as total scale, pH_T) and salinity in every second tank and by fixing samples for salinity, total alkalinity (TA) and dissolved inorganic carbon (DIC) for every tank. TA and DIC were later determined at the Alfred Wegener Institute (AWI), Bremerhaven, Germany.

The samples for pH_T were collected bubble free in 20-ml scintillation vials and measured within 1-2 hours of sampling with a spectrophotometer (Genesys 10SVIS) equipped with a single cell Peltier (SPG1A, both Thermo Scientific) using the indicator dye m-cresol purple (Sigma-Aldrich) at a constant temperature of 25ºC. The absorbance was measured at 730 nm, 578, and 434 nm before and after dye addition . The pH_T was calculated following SOP6b. Samples for TA were filtered through 0.6-µm filters (Poretics), fixed with $HgCl_2$ (end concentration ~120 mg/L) and stored in 150-mL borosilicate bottles. DIC samples were filtered through 0.2-µm cellulose-acetate filters into 5-mL borosilicate bottles, and also fixed with $HgCl_2$. TA was calculated from linear Gran plots (Gran 1952) after duplicate potentiometric titration (Brewer et al. 1986) using a TitroLine alpha plus (Schott Instruments, Mainz, Germany). Average precision was ± 5 µmol kg^{-1}. Certified Reference Materials (CRMs, Batch No. 54) supplied by A. Dickson (Scripps Institution of Oceanography, USA) was used as a control. Salinity was determined from TA samples using a conductivity instrument (3100 Yellow Springs Instruments). DIC was measured colorimetrically in duplicate with a TRAACS CS800 autoanalyzer (Seal, Mequon, USA) with a precision of ± 5 µmol kg^{-1}. CRM (Batch No. 54) supplied by A. Dickson was used as a calibration (see for methodological details). Averages of replicate measurements of TA and DIC are given.

The carbonate system was over-determined in half of the samples. Measured pH values were slightly higher than those calculated from TA and DIC, as is common , but were well correlated with them (pH_{Tcalc} = 1.0104 pH_{Tmeas}; r^2= 0.85, n=7). DIC and TA values were used to calculate the carbonate system with two exceptions. Two TA-samples contained precipitate of unknown origin and the replicate measurements of these showed high variability (> 5 µmol/kg). These two TA samples were not used, but instead TA was calculated from corrected pH and DIC.

Image analysis:

A MATLAB software developed for the supervised classification of images was used to determine the total area of settled aggregates from the photos of each tank . The different shades of coloring of the aggregates were first identified in each photo by the user. A statistical model was then used with this subset of data to assign every pixel of the image as belonging to an aggregate or not. The resulting black and white image was manually compared to the actual photo to determine accuracy and the total area of aggregates was then calculated by the software. In a second step, the mean area per aggregate was determined by circling 15 aggregates individually. This had to be done manually as aggregates on the photos often lay on top of each other. The average diameter per aggregate was then calculated from the mean area per aggregate assuming circles, independent of the actual shape. The average number of aggregates was then calculated from total and average aggregate area.

Sinking velocity:

The sinking velocities of ten randomly selected aggregates from each tank were measured in a 2-liter cylindrical settling column that was 8 cm wide in a temperature constant room . Each aggregate was allowed to sink out of the wide bore pipette into the settling column. The duration of the descent of the aggregate over a distance of 20 cm, from 6 cm below the surface to 6 cm above the bottom, was monitored using a stop watch. Just before this, the length and width of each aggregate were measured to the nearest mm under a dissecting scope. Aggregate volume was calculated based on an ellipsoid shape, assuming that width and depth were identical.

Particulate matter:

DW, POM, and PIM were determined from one duplicate set of pre-weighed, pre-combusted (450ºC for 4-6 hours) GF/F filters, and DW, POC, and PON from another. For the DW of all of these samples, the filters were reweighed after samples had been filtered on to them, salts had been rinsed away, and the filters dried at 60°C overnight. The PIM and POM filters were then combusted at 450ºC for 4-6 hours to remove organic matter and weighed a third time. POM was calculated by difference from the total DW and the PIM remaining after combustion. The filters for POC and PON were fumed to remove inorganic carbon and analyzed at the Analytical Laboratory of the Marine Science Institute at UCSB using an elemental analyzer (CEC 44OHA by Control Equipment Corp). This yielded a total of 4 replicate DW measurements per sample and 2 replicates each for POC, PON, POM, and PIM.

TEP was measured colorimetrically on 3-4 replicates per sample by gentle filtration onto 0.4 µm PC filters (Poretics) and staining with Alcian Blue . The dye solution was calibrated using Gum Xanthan and TEP are expressed as Gum Xanthan equivalents per liter (GXeq.L^{-1}).

Dissolved matter:

Concentrations of NO_3+NO_2, PO_4 and $Si(OH)_4$ in the initial phytoplankton-detritus mixture were measured by simultaneous flow injection analysis (QuickCem 8000, Lachat Instruments) in the Analytical Laboratory of the Marine Science Institute at UCSB from pre-filtered frozen samples.

DOC was sampled and measured as in . Replicate samples were filtered inline through acid-cleaned polycarbonate filter holders holding combusted GF/F filters into acid-cleaned glass and LDPE containers. The filtered samples were immediately frozen at -20°C and later analyzed via high temperature combustion on a modified Shimadzu TOC-V analyzers in the Carlson lab at UCSB.

The matrix of 14 treatments/ tanks in the experiment

Illite additions (µg/L)	**pCO_2 treatment (in µatm)**		
	Ambient	**Ambient + 260**	**Ambient +520 ppm**
0	1	6	10
500	2	7	11
2000	3	8	12
6000	4	Data missing	13
10000	5	9	14