

Supplement

1. Material and Methods

1.1 Media preparation

Sterile-filtered (0.2 μm) North Sea seawater (NSW) containing 7.2 $\mu\text{mol L}^{-1}$ silicate was enriched with vitamins and trace metals according to f/2 media (Guillard and Ryther, 1962) as well as with nitrate and phosphate. Final values of nitrate and phosphate were 111.5 $\mu\text{mol L}^{-1}$ and 5.85 $\mu\text{mol L}^{-1}$, respectively. Nutrients were measured colourimetrically using a continuous flow analyzer (Evolution III, Alliance Instruments, Salzburg, Austria). The salinity was 32.38 ± 0.003 (measured with a Guildline Autosol 8400B, Ontario, Canada). Inorganic artificial seawater (ASW) set to a salinity of 35 was prepared from milliQ water and dried salts as described by Grasshoff et al. (1999).

1.2 C_T adjustments at constant A_T

Adjustments of dissolved inorganic carbon (C_T) were conducted at $15 \pm 0.2^\circ\text{C}$ in 2 L borosilicate DURAN bottles equipped with glass frits for aeration. The media were sparged continuously with humidified, 0.2 μm -filtered air of different partial pressures of CO_2 (180 and 1000 μatm , each in triplicates). Gas flow rates were $130 \pm 10 \text{ mL min}^{-1}$. Gas mixtures were generated using a custom-made gas flow controller. CO_2 -free air ($<1 \text{ ppm CO}_2$; Dominick Hunter, Willich, Germany) was mixed with pure CO_2 (Air Liquide Deutschland, Düsseldorf, Germany) by a mass flow controller based system (CGM 2000 MCZ Umwelttechnik, Bad Nauheim, Germany). The CO_2 concentration was regularly measured with a non-dispersive infrared analyzer system (LI6252, LI-COR Biosciences, Bad Homburg, Germany) calibrated with CO_2 -free air and purchased gas mixtures of 150 ± 10 and $1000 \pm 20 \text{ ppm CO}_2$ (Air Liquide Deutschland, Düsseldorf, Germany). Seawater samples were taken after 48 hours to ensure equilibration (Fig. 2, supplement).

1.3 A_T adjustments at constant C_T

Total alkalinity (A_T) was adjusted by addition of calculated amounts of HCl or NaOH (1N Titrisol, Merck, Darmstadt, Germany) to seawater of known C_T . The manipulated media were stored in 2 L borosilicate DURAN bottles, which were sealed immediately with Teflon-lined screw caps without headspace to avoid CO_2 exchange with the atmosphere.

1.4 Sampling and measurements

Samples for A_T were 0.6 μm -filtered, stored in 150 mL borosilicate bottles at 3°C and analysed within a week. A_T was estimated from open-cell duplicate potentiometric titration at $22 \pm 2^\circ\text{C}$ calculated with modified Gran plots (Brewer et al., 1986) using a TitroLine alpha plus system (Schott Instruments, Mainz, Germany). The calculated A_T values were corrected for offsets between $\text{CRM}_{\text{measured}}$ and $\text{CRM}_{\text{defined}}$ ($-13 \pm 5 \mu\text{mol kg}^{-1}$, $n=10$, reproducibility; Certified Reference Materials Batch No. 54, Scripps Institution of Oceanography, USA). An internal North Sea seawater standard was additionally measured.

C_T samples were filtered through 0.2 μm cellulose-acetate filters and stored in 5 mL gas-tight borosilicate bottles at 3°C. As samples were analysed within one week, potential decomposition of organic matter during this period is small and, in any case, would have increased C_T values rather than being suitable to explain the discrepancies described. C_T was measured colourimetrically in triplicate with a QuAatro autoanalyzer (Seal, Mequon, USA). The analyser was calibrated with NaHCO_3 solutions (with a salinity of 35, achieved by addition of NaCl) with concentrations ranging from 1800 to 2300 $\mu\text{mol } C_T \text{ kg}^{-1}$. CRMs were used for corrections of errors in instrument performance (baseline drift etc.). On average, the CRMs were measured with an offset of $3 \pm 5 \mu\text{mol kg}^{-1}$ ($n=20$, reproducibility) from the assigned value. Shifts in C_T due to CO_2 exchange were limited by opening the storage vials less than one minute prior to each measurement.

Seawater pH values were determined by two different approaches, potentiometrically as well as spectrophotometrically. In case of the NSW samples, pH_{total} was determined spectrophotometrically using *m*-cresol purple (Merck KGaG, Darmstadt, Germany; Clayton and Byrne, 1993, Dickson et al., 2007) using a temperature-controlled Cary 4000 UV-VIS-NIR spectrophotometer (Varian, Palo Alto, USA). The spectrophotometric pH_{total} was measured in technical duplicates at $25 \pm 0.1^\circ\text{C}$, temperature-corrected and recalculated to 15.0°C using CO_2sys (Pierrot et al., 2006). The performance of the spectrophotometer used for the pH_{total} measurements (Cary 4000, Varian) was validated complying with the US Pharmacopeia requirements. Absorption measurements were corrected for background absorption (Dickson et al., 2007) and pH changes due to dye addition (calculated from the average effect of a second dye addition to every sample). Furthermore, the average values of CRM measurements (Tris-based pH reference materials) at the beginning and the end of one measuring run were used to correct the sample values. On average, the CRMs were measured with an offset of -0.003 ± 0.003 ($n=7$, repeatability at $\text{pH} = 8.1$) from the assigned value. Carbonate chemistry calculations were based on total scale pH measurements. For the

potentiometric measurement (no replication), the glass reference electrode (IOLine, Schott Instruments) was calibrated using two buffers traceable back to NIST standards prior to every set of measurements. The pH of NSW samples was thus measured on the NBS scale (pH_{NBS}). These potentiometric measurements were performed at $15 \pm 0.2^\circ\text{C}$, temperature-corrected and reported for 15.0°C using CO₂sys. The repeatability was ± 0.02 pH units ($n=30$). In the case of the ASW samples, seawater pH on the total scale (pH_{total}) was measured potentiometrically. The measured pH_{NBS} values were converted to the total scale using respective CRMs after determining the offset of measured and assigned pH value (Tris-based pH reference material, Batch No. 2, Scripps Institution of Oceanography, USA).

CO₂ concentrations were determined at $15 \pm 0.2^\circ\text{C}$ in technical triplicates using a membrane-inlet mass spectrometer (MIMS; Tortell, 2005), which consists of a thermostated cuvette being connected to a sector field multi-collector MS (Isoprime; GV Instruments, England). The MIMS was calibrated for CO₂ by injections of known amounts of NaHCO₃ into 8 ml of 0.2 N HCl (Schulz et al., 2006). The CO₂ baseline was determined by addition of 20 μL 10 N NaOH. CO₂ concentrations were measured with a reproducibility of $\pm 0.13 \mu\text{mol kg}^{-1}$ ($n = 15$).

1.5 Calculations of carbonate chemistry

Calculations were performed using the program CO₂sys (Pierrot et al., 2006) and verified with the MATLAB files based on Zeebe and Wolf-Gladrow (2001). The dissociation constants of carbonic acid of Mehrbach et al. (1973; refit by Dickson and Millero, 1987) were used for calculations. Dissociation constants for HSO₄ were taken from Dickson (1990). CO₂ concentrations obtained with MIMS were converted to $f\text{CO}_2$ by applying Henry's law (Weiss, 1974) and further converted to $p\text{CO}_2$ using CO₂sys.

In case of the study by Schneider & Erez (2008), nutrient concentrations were taken from Makey et al. (2009; $0.1 \mu\text{mol kg}^{-1}$ phosphate and $2 \mu\text{mol kg}^{-1}$ silicate). In case of the dataset from Thomsen et al. (2010), concentration of $2 \mu\text{mol kg}^{-1}$ phosphate and $3 \mu\text{mol kg}^{-1}$ silicate were assumed (Gerlach, 1990). In case of the dataset of Iglesias-Rodríguez et al. (2008), typical concentrations in (non-diatom) phytoplankton cultures were assumed ($8 \mu\text{mol kg}^{-1}$ phosphate and $6 \mu\text{mol kg}^{-1}$ silicate).

Table 1. Measured carbonate chemistry parameters (pH, C_T , A_T and $[CO_2]$) and resulting pCO_2 values calculated from different input parameters using the constants of Mehrbach et al. (1973, refit by Dickson and Millero, 1987) at 15°C. Errors denote standard deviation of technical replicates.

		measured					calculated			
		pH _{NBS}	pH _{total}	C _T (μmol kg ⁻¹)	A _T (μmol kg ⁻¹)	CO ₂ (μmol kg ⁻¹)	pCO ₂ (MIMS)	pCO ₂ (A _T ;C _T)	pCO ₂ (A _T ;pH)	pCO ₂ (C _T ;pH)
NSW C _T manipulation	low pCO ₂	8.38	8.252 ± 0.005	1945 ± 7	2388 ± 2	8.34 ± 0.14	205	143	241	227
		8.39	8.319 ± 0.001	1946 ± 7	2385 ± 3	7.82 ± 0.23	203	145	197	190
		8.39	8.305 ± 0.002	1946 ± 4	2383 ± 3	7.73 ± 0.23	219	146	202	195
	high pCO ₂	7.91	7.735 ± 0.001	2254 ± 1	2396 ± 1	39.31 ± 0.44	1042	662	934	913
		7.88	-	2254 ± 1	2386 ± 1	38.10 ± 0.02	1000	702	-	-
		7.89	7.712 ± 0.000	2256 ± 6	2388 ± 3	42.59 ± 0.02	1117	703	1003	981
NSW A _T manipulation	low pCO ₂	8.46	8.313 ± 0.003	2211 ± 6	2643 ± 1	9.06 ± 0.07	259	191	226	222
		8.48	8.318 ± 0.012	2217 ± 6	2642 ± 0	9.10 ± 0.04	268	196	219	217
		8.48	8.317 ± 0.000	2220 ± 4	2640 ± 3	8.60 ± 0.08	238	199	219	217
	high pCO ₂	7.72	7.616 ± 0.004	2186 ± 2	2276 ± 1	47.48 ± 1.63	1202	881	1193	1172
		7.81	7.610 ± 0.004	2180 ± 4	2278 ± 3	47.51 ± 0.23	1247	831	1224	1197
		7.79	7.617 ± 0.013	2186 ± 1	2276 ± 1	47.89 ± 0.02	1257	881	1193	1172
ASW A _T manipulation	low pCO ₂	-	8.20	2192 ± 1	2612 ± 0	-	-	205	298	286
		-	8.20	2204 ± 2	2605 ± 3	-	-	220	297	288
		-	8.19	2206 ± 0	2597 ± 2	-	-	227	305	296
	intermed. pCO ₂	-	7.88	2184 ± 1	2390 ± 1	-	-	461	644	628
		-	7.88	2199 ± 6	2391 ± 2	-	-	500	644	632
		-	7.88	2199 ± 1	2389 ± 1	-	-	505	643	632
	high pCO ₂	-	7.60	2087 ± 6	2173 ± 1	-	-	865	1183	1161
		-	7.49	2119 ± 4	2172 ± 2	-	-	1121	1547	1519
		-	7.47	2122 ± 6	2172 ± 3	-	-	1149	1624	1592

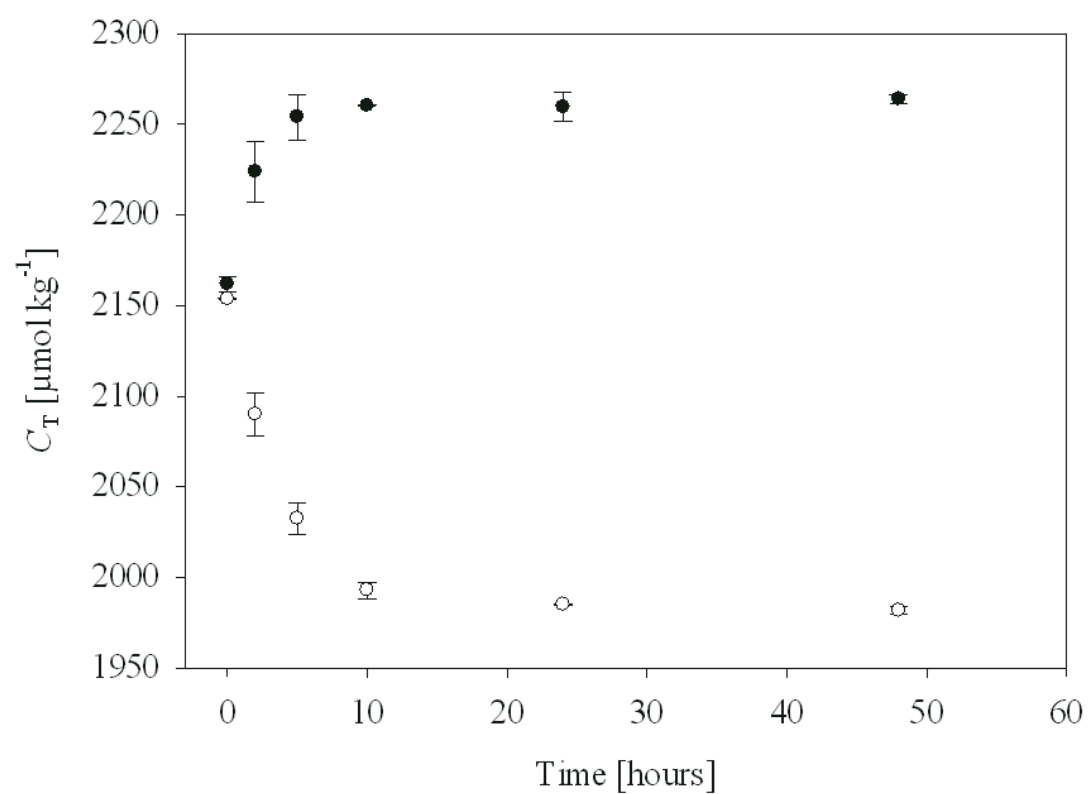


Figure 2. Equilibration kinetics in C_T manipulation experiments at 15°C represented by changes in C_T at low (open circles) and high $p\text{CO}_2$ (filled circles). Flow rates of equilibration gasses were $130 \pm 10 \text{ mL min}^{-1}$.

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