

1 Supplement

2

3 Material and Methods

4 1. Media preparation

5 Sterile-filtered (0.2 μm) North Sea seawater (NSW) containing 7.2 $\mu\text{mol L}^{-1}$ silicate was
6 enriched with vitamins and trace metals according to f/2 media (Guillard and Ryther, 1962) as
7 well as with nitrate and phosphate. Final values of nitrate and phosphate were 111.5 $\mu\text{mol L}^{-1}$
8 and 5.85 $\mu\text{mol L}^{-1}$ respectively. Nutrients were measured colourimetrically using a continuous
9 flow analyzer (Evolution III, Alliance Instruments, Salzburg, Austria). The salinity was
10 32.380 \pm 0.003 (measured with a Guildline Autosal 8400B, Ontario, Canada). Inorganic
11 artificial seawater (ASW) was prepared from milliQ water and salts as described by Grasshoff
12 et al. (1999), set to a salinity of 35.

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14 2. C_T Adjustments at constant A_T

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

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28 3. Alkalinity adjustments at constant C_T

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

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1 4. Sampling and measurements

2 Samples for total alkalinity (A_T) were 0.6 μm -filtered, stored in 150 mL borosilicate bottles at
3 3°C and analysed within a week. A_T was estimated from open-cell duplicate potentiometric
4 titration at $22\pm 2^\circ\text{C}$ calculated with modified Gran plots (Brewer et al., 1986) using a
5 TitroLine alpha plus (Schott Instruments, Mainz, Germany). The calculated A_T values were
6 corrected for offsets between $\text{CRM}_{\text{measured}}$ and $\text{CRM}_{\text{defined}}$ (on average $-13 \mu\text{mol kg}^{-1}$; Certified
7 Reference Materials Batch No. 54, Scripps Institution of Oceanography, USA). An internal
8 North Sea seawater standard was additionally measured. The corrected A_T value was always
9 $2445 \pm 5 \mu\text{mol kg}^{-1}$ (repeatability; $n = 10$).

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

21 Seawater pH values were determined by two different approaches, potentiometrically as
22 well as spectrophotometrically. In case of the NSW samples, pH_{total} was determined
23 spectrophotometrically using *m*-cresol purple (Merck KGaG, Darmstadt, Germany; Clayton
24 and Byrne, 1993, Dickson et al., 2007) using a temperature-controlled Cary 4000 UV-VIS-
25 NIR spectrophotometer (Varian, Palo Alto, USA). The spectrophotometric pH_{total} was
26 measured in technical duplicates at $25 \pm 0.1^\circ\text{C}$, temperature-corrected and recalculated to
27 15.0°C using CO_2sys . The performance of the spectrophotometer used for the pH_{total}
28 measurements (Cary 4000, Varian) was validated complying with the US Pharmacopeia
29 requirements. Absorption measurements were corrected for background absorption (Dickson
30 et al., 2007) and pH changes due to dye addition (calculated from the average effect of a
31 second dye addition to every sample). Furthermore, the average values of CRM
32 measurements (Tris-based pH reference materials) at the beginning and the end of one
33 measuring run were used to correct the sample values. On average, the CRMs were measured
34 with an offset of -0.003 ± 0.003 from the assigned value (repeatability at $\text{pH} = 8.1$; $n=7$).

1 Carbonate chemistry calculations were based on total scale pH measurements. For the
2 potentiometric measurement (no replication), the glass reference electrode (IOLine, Schott
3 Instruments) was calibrated using two buffers traceable back to NIST standards prior to every
4 set of measurements. The pH of NSW samples was thus measured on the NBS scale (pH_{NBS}).
5 These potentiometric measurements were performed at $15 \pm 0.2^\circ\text{C}$, temperature-corrected and
6 reported for 15.0°C using CO_2sys (Pierrot et al., 2006). The repeatability was ± 0.02 pH units
7 ($n=30$). In the case of the ASW samples, seawater pH on the total scale (pH_{total}) was measured
8 potentiometrically. The measured pH_{NBS} values were converted to the total scale using
9 respective CRMs after determining the offset of measured and assigned pH value (Tris-based
10 pH reference material, Batch No. 2, Scripps Institution of Oceanography, USA).

11 CO_2 concentrations were determined at $15 \pm 0.2^\circ\text{C}$ in technical triplicates using a
12 membrane-inlet mass spectrometer (MIMS; Tortell, 2005), which consists of a thermostated
13 cuvette being connected to a sector field multi-collector MS (Isoprime; GV Instruments,
14 England). The MIMS was calibrated for CO_2 by injections of known amounts of NaHCO_3
15 into 8 ml of 0.2 N HCl (Schulz et al., 2006). The CO_2 baseline was determined by addition of
16 20 μL 10 N NaOH. CO_2 concentrations were measured with a reproducibility of $\pm 0.13 \mu\text{mol}$
17 kg^{-1} ($n = 15$).

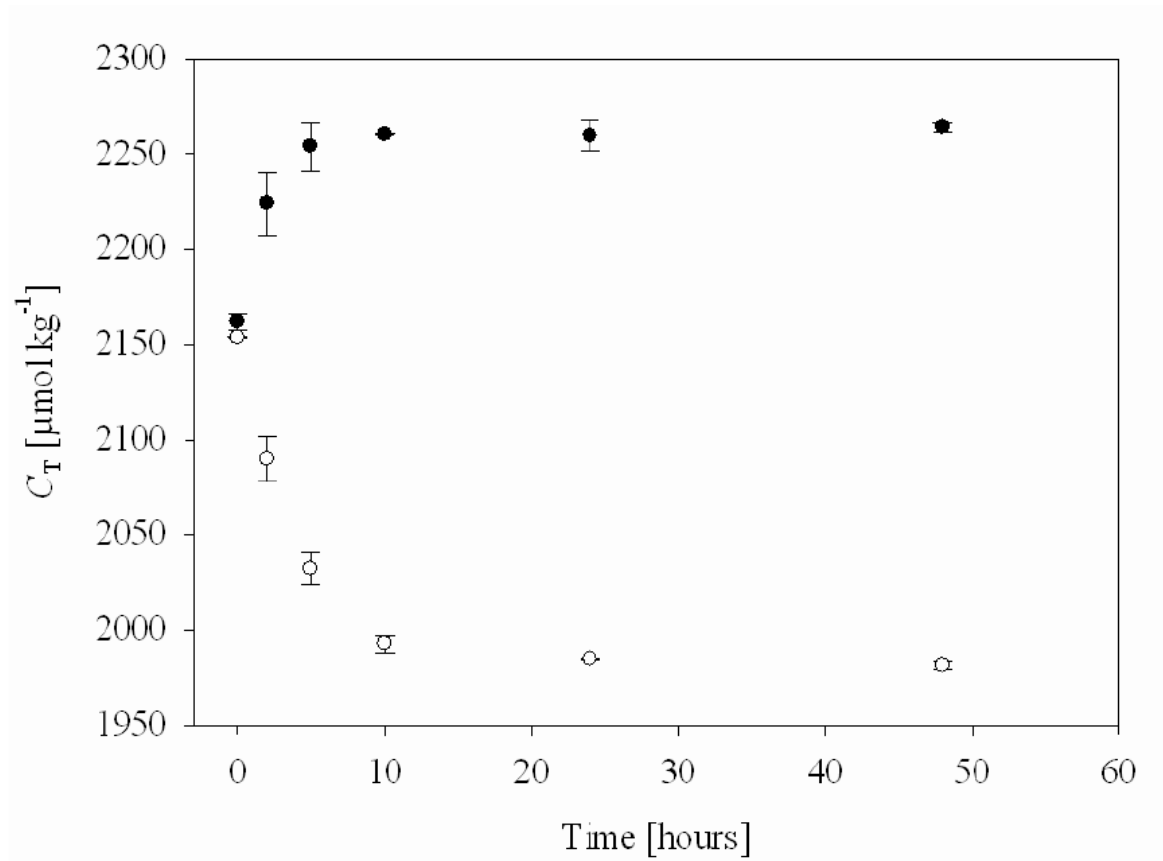
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19 **5. Calculations of carbonate chemistry**

20 Calculations were performed using the program CO_2sys (Pierrot et al., 2006) and verified with
21 the MATLAB files based on Zeebe and Wolf-Gladrow (2001). The dissociation constants of
22 carbonic acid of Mehrbach et al. (1973; refit by Dickson and Millero, 1989,) were used for
23 calculations. Dissociation constants for HSO_4 were taken from Dickson (1990). CO_2
24 concentrations obtained with MIMS were converted to $f\text{CO}_2$ by applying Henry's law (Weiss,
25 1974) and further converted to $p\text{CO}_2$ using CO_2sys (Pierrot et al., 2006).

1 **Table 2.** Measured carbonate chemistry parameters (pH, C_T , A_T and $[CO_2]$) and resulting pCO_2 values calculated from different input parameters
 2 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 ($\mu\text{mol kg}^{-1}$)	A_T ($\mu\text{mol kg}^{-1}$)	CO_2 ($\mu\text{mol kg}^{-1}$)	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	240	226
		8.39	8.319 ± 0.001	1946 ± 7	2385 ± 3	7.82 ± 0.23	203	145	198	191
		8.39	8.305 ± 0.002	1946 ± 4	2383 ± 3	7.73 ± 0.23	219	146	206	198
	high pCO_2	7.91	7.735 ± 0.001	2254 ± 1	2396 ± 1	39.31 ± 0.44	1042	660	945	924
		7.88	-	2254 ± 1	2386 ± 1	38.10 ± 0.02	1000	701	-	-
		7.89	7.712 ± 0.000	2256 ± 6	2388 ± 3	42.59 ± 0.02	1117	702	998	976
NSW A_T manipulation	low pCO_2	8.46	8.313 ± 0.003	2211 ± 6	2643 ± 1	9.06 ± 0.07	259	191	224	220
		8.48	8.318 ± 0.012	2217 ± 6	2642 ± 0	9.10 ± 0.04	268	196	221	218
		8.48	8.317 ± 0.000	2220 ± 4	2640 ± 3	8.60 ± 0.08	238	199	221	219
	high pCO_2	7.72	7.616 ± 0.004	2186 ± 2	2276 ± 1	47.48 ± 1.63	1202	881	1205	1183
		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	1202	1181
ASW A_T manipulation	low pCO_2	-	8.20	2192 ± 1	2612 ± 0	-	-	204	295	283
		-	8.20	2204 ± 2	2605 ± 3	-	-	220	301	291
		-	8.19	2206 ± 0	2597 ± 2	-	-	228	304	295
	intermed. pCO_2	-	7.88	2184 ± 1	2390 ± 1	-	-	462	649	632
		-	7.88	2199 ± 6	2391 ± 2	-	-	498	649	636
		-	7.88	2199 ± 1	2389 ± 1	-	-	504	651	639
	high pCO_2	-	7.60	2087 ± 6	2173 ± 1	-	-	875	1188	1166
		-	7.49	2119 ± 4	2172 ± 2	-	-	1127	1559	1530
-	7.47	2122 ± 6	2172 ± 3	-	-	1159	1607	1577		



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

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