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Technical Note: A minimally invasive experimental system for pCO_2 manipulation in plankton cultures using passive gas exchange (atmospheric carbon control simulator)

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Gas Mixing System: Air is supplied by an oil free compressor (Powerex SES03 Oil free rotary scroll) with a 250 litre storage tank (Manchester Tank). It is possible to operate the system with small compressors (e.g. Dewalt 1.9 HP, oil free D55168) however reliability is an issue and the investment in a compressor rated for continuous operation is recommended. The storage tank is supplied with an automatic drain valve (Posi-drain). Again, manual draining of the storage tank is possible, but not recommended. Dry air is then passed through continuously regenerating CO_2 adsorbers (Puregas CAS2-11) at about 90-100 psi. A high precision regulator steps down the pressure to 30 psi as the CO_2 free air enters the MFCs (Sierra Instruments SmarTrak). The MFCs for air are capable of regulating flows up to 20 L min^{-1}. A second set of MFCs receive pure research grade CO_2 at 30 psi and regulate flows up to 25 cc min^{-1}. The two gas streams are mixed, and pass through a back pressure regulator (ControlAir 700BP) which ensures that the pressure on the outlet of the MFCs remains at a constant 15 psi.

Carbonate Verification: Samples for spectrophotometric pH are collected via 30 ml syringe to minimize gas exchange. They are very gently filtered through a pre-rinsed GFF filter (Whatman) because some cultures are optically dense enough to negatively impact spectrophotometric results. The first 5 ml of filtrate are discarded, and samples are syringe filtered slowly (about 10 ml/min) to minimize cell lysis. 15 ml vials are overflowed, again to minimize gas exchange in the sample. Recognized filtering methods via peristaltic pump (Bockmon and Dickson, 2014) are designed for large field samples and are not practical for a large number of small volume samples. The vials are brought to temperature (25 °C) in a temperature controlled water bath. Each sample is transferred via syringe to a water jacketed temperature controlled 5 cm cuvette, which is rinsed and then overflowed with the sample. The optical surfaces of the cuvette are cleaned with ethanol and lens paper. All other aspects of the measurement are according to the m-cresol blue method, with 20 μL of indicator used to achieve the appropriate level of absorbance on the Agilent 8453A UV-VIS diode array spectrophotometer (Dickson et al., 2007). This 5 cm cuvette was found to be a good compromise between the high precision but also high volume needed to use a 10 cm cuvette and the difficult temperature control and repeatability for 1 cm cuvettes. The temperature stability of the water jacket outweighs the negative impact of increased sample handling necessary to produce high quality data.

DIC is determined using the Apollo Scitech DIC analyzer (AS-C3). A standard curve is run each day, using Dickson Certified Reference Material (Batch 148 and 152 for ACCS 1-4), corrected for density at ambient temperature. For each sample, 3 to 5 aliquots, each 0.75ml, are acidified with 10% phosphoric acid, degassed and analyzed for CO_2 via Licor 7700 NDIR instrument. When analyses match to

within 0.2%, the next sample is run. Density based on temperature and salinity allow the results in µmol L^{-1} from this volume based system to be converted to µmol kg^{-1}.

Samples for total alkalinity are preserved in 100 ml screw cap glass bottles with Teflon lined caps by the addition of 20 ul of saturated mercuric chloride. Total alkalinity is determined via open cell gran titration with 0.1 N HCl similar to Dickson (2007) using a Metrohm 888 Titrando and PT1000 electrode. Temperature is controlled using a water jacketed beaker and burette. At least 2 titrations of Dickson Certified Reference Material (Batch 148 and 152 for ACCS 1-4) are carried out before samples. If resulting alkalinities are both within 5 µmol kg^{-1} of the certified value then samples are analysed, with additional reference material titrations periodically. Other carbonate parameters are calculated with CO2sys (Lewis and Wallace, 1998) using the constants of Mehrbach (1987) refit by Dickson (1987).

Table S1: Complete carbonate parameters for experiments 1-4. Values are mean across all days followed by the standard deviation in parentheses. Samples were analysed in triplicate for pH and DIC on 2-10 days during each experiment, in rotation with development jars, and other samples of interest. CO_2 partial pressure, total alkalinity and calcite saturation state were calculated using CO2sys. Alkalinity data directly titrated are available on the BCO-DMO data repository. Under type, algae refers to the phytoplankton. Species are listed in Table 2. The copepods are adult females.

Experiment	Type	Treatment	Salinity (ppt)	*p*CO2 (uatm)	pH (total)	Total Carbon (umol/kg)	Total Alkalinity (ueq/kg)	Ω Calcite	n
1	Algae	A	31.7 (0.7)	317 (27)	8.11 (0.03)	1925 (17)	2123 (18)	3.6 (0.2)	20
		M	31.9 (0.7)	549 (94)	7.90 (0.06)	1977 (23)	2098 (31)	2.4 (0.3)	21
		H	31.4 (0.5)	762 (159)	7.77 (0.07)	2006 (28)	2082 (41)	1.8 (0.2)	18
	Equilibration tanks	A	31.2 (0.7)	417 (12)	8.00 (0.01)	1916 (17)	2063 (21)	2.8 (0.1)	31
		M	31.3 (0.7)	811 (36)	7.73 (0.02)	1996 (23)	2059 (25)	1.7 (0.1)	29
		H	31.2 (0.8)	1134 (132)	7.60 (0.06)	2034 (22)	2060 (30)	1.3 (0.2)	33
	Copepods	A	31.1 (0.7)	596 (66)	7.86 (0.04)	1966 (30)	2066 (28)	2.1 (0.2)	27
		M	31.1 (0.8)	1011 (89)	7.64 (0.03)	2015 (32)	2053 (30)	1.4 (0.1)	27
		H	31.2 (0.8)	1307 (119)	7.54 (0.04)	2036 (30)	2045 (27)	1.1 (0.1)	26
2	Algae	A	32.5 (1.3)	381 (19)	7.85 (0.02)	1947 (42)	2121 (55)	3.2 (0.2)	18
		M	30.8 (0.4)	415 (16)	7.8 (0.02)	1851 (33)	1990 (36)	2.6 (0.1)	6
		H	31.8 (0.8)	519 (21)	7.73 (0.02)	1937 (25)	2056 (27)	2.4 (0.1)	18
	Equilibration tanks	A	32.7 (1.2)	1079 (39)	7.46 (0.02)	2066 (53)	2104 (60)	1.4 (0.1)	17
		M	31.0 (0.0)	1153 (44)	7.41 (0.01)	1967 (25)	1986 (26)	1.2 (0.)	6
		H	31.7 (1.2)	1239 (45)	7.4 (0.02)	2033 (19)	2049 (23)	1.2 (0.1)	18
	Copepods	A	32.6 (1.2)	777 (29)	7.58 (0.01)	2032 (56)	2107 (64)	1.8 (0.1)	18
		M	31.4 (0.5)	844 (47)	7.53 (0.02)	1953 (36)	2007 (35)	1.5 (0.)	8
		H	31.5 (1.2)	930 (36)	7.51 (0.02)	2003 (21)	2050 (25)	1.5 (0.1)	18

Experiment	Type	Treatment	Salinity (ppt)	*p*CO2 (uatm)	pH (total)	Total Carbon (umol/kg)	Total Alkalinity (ueq/kg)	Ω Calcite	n
3	Algae	A	31.7 (0.7)	317 (27)	8.11 (0.03)	1925 (17)	2123 (18)	3.6 (0.2)	20
		M	31.9 (0.7)	549 (94)	7.9 (0.06)	1977 (23)	2098 (31)	2.4 (0.3)	21
		H	31.4 (0.5)	762 (159)	7.77 (0.07)	2006 (28)	2082 (41)	1.8 (0.2)	18
	Equilibration tanks	A	31.2 (0.7)	417 (12)	8.00 (0.01)	1916 (17)	2063 (21)	2.8 (0.1)	31
		M	31.3 (0.7)	811 (36)	7.73 (0.02)	1996 (23)	2059 (25)	1.7 (0.1)	29
		H	31.2 (0.8)	1134 (132)	7.60 (0.06)	2034 (22)	2060 (30)	1.3 (0.2)	33
	Copepods	A	31.1 (0.7)	596 (66)	7.86 (0.04)	1966 (30)	2066 (28)	2.1 (0.2)	27
		M	31.1 (0.8)	1011 (89)	7.64 (0.03)	2015 (32)	2053 (30)	1.4 (0.1)	27
		H	31.2 (0.8)	1307 (119)	7.54 (0.04)	2036 (30)	2045 (27)	1.1 (0.1)	26
4	Algae	A	33 (1.3)	381 (19)	8.04 (0.02)	1947 (42)	2121 (55)	3.2 (0.2)	18
		M	30.8 (0.4)	415 (16)	7.99 (0.02)	1851 (33)	1990 (36)	2.6 (0.1)	6
		H	31.8 (0.8)	519 (21)	7.91 (0.02)	1937 (25)	2056 (27)	2.4 (0.1)	18
	Equilibration tanks	A	32.6 (1.2)	777 (29)	7.76 (0.02)	2032 (56)	2107 (64)	1.8 (0.1)	18
		M	31.4 (0.5)	844 (47)	7.71 (0.02)	1953 (36)	2007 (35)	1.5 (0.)	8
		H	31.5 (1.2)	930 (36)	7.68 (0.02)	2003 (21)	2050 (25)	1.5 (0.1)	18
	Copepods	A	32.7 (1.2)	1079 (39)	7.62 (0.02)	2066 (53)	2104 (60)	1.4 (0.1)	17
		M	31. (0.)	1153 (44)	7.58 (0.02)	1967 (25)	1986 (26)	1.2 (0.)	6
		H	31.7 (1.2)	1239 (45)	7.56 (0.02)	2033 (19)	2049 (23)	1.2 (0.1)	18

Table S2: Mean pH (standard deviation) of cultures of Emiliania huxleyi with varying volumes in the flask (n=3), resulting in surface area to volume ratios of 162, 161 and 90 cm^2/L for 500, 700 and 900 ml respectively. Cell density did not vary significantly between treatments.

Sample	Days of Growth									
	1		2		3		4		5	
Blank	7.71	(0.01)	7.72	(0.02)	7.76	(0.00)	7.72	(0.01)	7.75	(0.00)
500 ml	7.76	(0.01)	7.82	(0.01)	7.85	(0.01)	7.93	(0.01)	7.97	(0.01)
700 ml	7.77	(0.02)	7.8167	(0.02)	7.87	(0.01)	7.98	(0.01)	8.09	(0.01)
900 ml	7.76	(0.01)	7.80	0.01	7.88	(0.01)	8.03	(0.03)	8.18	(0.02)

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