Carbonate System Sampling in Zooplankton Hatching Experiments 6, 7, and 8 of the Project: Ocean Acidification impacts on Zooplankton in Northern Oceans.

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Summary: As part of collaborative research between Dr. John P. Christensen and Dr. Jeffrey A. Runge, funded by the research grant 1041081 under the Ocean Acidification Program of the National Science Foundation, experiments were conducted to test whether reduced pH of the ocean waters would negatively affect the hatching success of several zooplankton species found in northern ocean waters. These experiments were performed at the Darling Marine Center of the University of Maine, using local seawaters (salinities > 30). Each experiment consisted of incubating fresh zooplankton eggs in several 20 L seawater tanks at constant temperature for periods of up to 6 days. Each tank was maintained at fixed pH, and each experiment consisted of several tanks which covered a range of pH values at and below that of natural seawater. The measure of hatching success was the number of nauplii which were produced from the total number of incubated eggs. During the incubation, waters were extracted to measure carbonate system parameters and other key concentrations (titration alkalinity, total carbon dioxide, salinity, nitrate plus nitrite, ammonium, inorganic phosphate, and dissolved silicate), which were used to calculate the real pH of seawater in the incubation tanks. Analysis of these chemistry samples was performed at the Green Eyes LLC laboratory. This report briefly describes the experiments and details the analytical methods used for the seawater chemistries. Results from the incubation experiments numbered 6 through 8 are listed.

keywords: seawater carbon dioxide, ocean acidification, CO_2 methods, zooplankton, pH

Carbonate System Sampling in Zooplankton Hatching Experiments as part of Ocean Acidification impacts on Zooplankton in Northern Oceans: Experiments 6 through 8.

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METHODS and RESULTS

Hatching Experiments: The details of the preparation and execution of the hatching experiments are being described in several reports (Preziosi, 2012; Runge, Preziosi, and Christensen, in prep.). In brief, female zooplankton, freshly caught or reared in the lab with plentiful food, were maintained in a healthy condition and their released eggs separated. When the numbers of eggs required in an experiment were sufficient, the eggs were sorted into individual hatching dishes, each containing natural seawater and 30 eggs. Local seawaters were collected, filtered and used to fill several 20 L hatching tanks. Each tank was consisted of a polycarbonate tank and lid, and an aeration tube into which was bubbled a premixed gas, consisting of 20% oxygen, the preselected concentration of CO_2 (ranging from ambient local atmospheric levels to 50000 ppm in the dry gas), and the remainder nitrogen gas (N_2). Experiment 7 differed in that bubbling occurred via two premade gases, one of 917 ppm CO_2 as used in the other experiments, and one of 50000 ppm CO_2 in nitrogen. In Experiment 7, the flows of each were set after initial equilibration so that the desired pH of about 6 was maintained throughout the experiment. A siphon mounted within the tank allowed for sampling of the waters without opening the tank. A 1-inch diameter hole in the tank lid, normally stoppered, allowed for immersion of the pH and temperature electrodes occasionally throughout the incubation. After the pH stabilized to its quasi-equilibrium value for the bubbled gas, several hatching dishes containing the eggs were immersed in the tank. The tank was closed and hatching allowed to proceed. At an appropriate time, all hatching dishes were removed for determination of hatching success and the experiment then ended. During the incubation, either once (for incubation times less than 2 days) or twice, at the beginning and near the end of the longer 4-6 day experiments, about 2 liters of tank water were withdrawn for collection of the chemistry samples, as described below. The chemical results were used to determine the tank's pH.

Sea waters used in these hatching experiments were collected from nearby the University of Maine's Ira C. Darling Marine Center in Walpole Maine. The sampling site, DMC-2, is just off shore of the mouth of the Damariscotta River within 1 minute of latitude or longitude of 43.800°N and 69.550°W (-69.550°E). The sampling site has a water depth of approximately 110 m. The site, DMC, is from the dock area at the Darling Center (43.925°N and 69.567°W). The Damariscotta River has relatively low fresh water output so salinities are generally greater than 29 at all locations south of the Darling Marine Center. The specific depths from which waters for each experiment were collected are provided in the following table. Waters were collected by immersible pump, and filtered upon return to the Darling Marine Center. Then the waters were subdivided between the experimental tanks and equilibration to the experimental temperature and to the bubbled artificial air with pre-selected CO_2 concentrations were begun. All dates and times in this file and in the comma separated value files are in local time (UTC-4 at this time of year).

EXPERIMENT #	DATAFILE	SAMPLING DATE	SITE	SAMPLING DEPTH
#	name	(yyyy-mm-dd)	name	(m)
6	HDAT06-4.csv	2011-07-07	DMC-2	42
7	HDAT07-4.csv	2011-07-21	DMC-2	40
8	HDAT08-4.csv	2011-09-27	DMC	1

Chemistry: At each sampling event, about 2 L of the tank waters were siphoned for samples of salinity, titration alkalinity (TA), total carbon dioxide (TCO_2), and nutrients. The details of this sampling and the subsequent analysis is described in Christensen (2012). To calculate pH from these data, we used the carbonate equilibration model, CO2SYS (Lewis and Wallace, 1995; DOE, 1994). This employs the equilibration coefficients of Roy et al. (1993) for carbonate coefficients K1 and K2, of Weiss (1974) for carbon dioxide K0, of Dickson (1990b) for borate, of Dickson and Riley (1979) for fluoride, of Dickson (1990a) for sulfate, and of Millero (1995) for phosphate (kp1, kp2, kp3) and silicate. Seawater density at atmospheric pressure was that of UNESCO, 1981.

keywords: seawater carbon dioxide, ocean acidification, CO_2 methods, zooplankton, pH

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keywords: seawater carbon dioxide, ocean acidification, CO_2 methods, zooplankton, pH

DATA TABLES OF HATCHING EXPERIMENTS 6-8

TABLES 1-3. The sampling date for each set of samples was given as a 6 digit number (YYMMDD). Each sampling event collected chemical measurements from all tanks, where each tank was bubbled with a synthetic air mixture with a different CO_2 content. The key measurements were for temperature (TEMP in degrees C), salinity (SAL in parts per thousand by weight), alkalinity (ALKALIN in μmol of H^+ equivalents/kg of seawater), total carbon dioxide (TCO_2 in μmol of CO_2/kg of seawater), the nutrients nitrate plus nitrite (NO3+2KG), ammonium (NH4KG), dissolved inorganic phosphate (PO4KG), and dissolved silicate (SIKG). Nutrient concentrations were in units of μmol/kg. From these measurements, the total pH of the seawater and the calcite and aragonite saturation states (OMCA and OMAR respectively) were calculated. Since the saturation state is composed of a numerator and denominator of the same units, the numbers in these columns are unitless. The numbers, -9 or -99, indicated that no sample was collected or measured. The tables presented below are identical to spreadsheet readable versions which are comma separated value ASCII text files (file names: HDAT0x-4.csv, where x = 6 to 8). These files are found in the zip file HDAT6-8a.zip.

TABLE 1. Data from Experiment #6.

FILE:	HDAT06-4.CAL														
DATE:	26JAN2013														
BY:	JPC														
# OF ROWS:	25														
# OF COLS:	16														
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DATESMPL	HTCEXP	EVENT	TANK	GASCO2	TEMP	SAL	ALKALIN	TCO2	NO3+2KG	NH4KG	PO4KG	SIKG	PHTTL	OMCA	OMAR
---	---	---	---	[CO2]	---	---	---	---	---	---	---	---	---	---	---
YYMMDD	#	#	#	PPM	DEGC	GM/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	PHUNIT	ULESS	ULESS
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
110708	6	1	1	350	6	31.702	2241.3	2075.1	5.60	1.37	0.90	5.14	8.104	3.01	1.89
110708	6	1	2	913	6	31.652	2256.8	2101.2	5.27	1.41	0.94	5.52	8.076	2.86	1.80
110708	6	1	3	1474	6	31.638	2306.5	2157.6	5.16	1.65	0.96	5.61	8.053	2.80	1.76
110708	6	1	4	2327	6	31.670	2289.1	2185.7	5.31	1.38	0.95	5.33	7.930	2.16	1.35
110708	6	1	5	4894	6	31.665	2335.1	2293.4	5.37	1.48	0.94	5.43	7.394	0.66	0.41
110709	6	2	1	350	-9	-99	-99	-99	5.55	0.80	0.72	5.07	-99	-99	-99
110709	6	2	2	913	-9	-99	-99	-99	5.52	1.58	0.93	5.46	-99	-99	-99
110709	6	2	3	1474	-9	-99	-99	-99	5.31	1.81	0.88	5.50	-99	-99	-99
110709	6	2	4	2327	-9	-99	-99	-99	5.20	1.53	0.93	5.25	-99	-99	-99
110709	6	2	5	4894	-9	-99	-99	-99	5.20	1.39	0.87	5.37	-99	-99	-99
110710	6	3	1	350	-9	-99	-99	-99	4.10	0.24	0.44	5.02	-99	-99	-99
110710	6	3	2	913	-9	-99	-99	-99	5.12	1.27	0.80	5.39	-99	-99	-99
110710	6	3	3	1474	-9	-99	-99	-99	5.02	1.01	0.75	5.59	-99	-99	-99
110710	6	3	4	2327	-9	-99	-99	-99	5.13	1.33	0.83	5.25	-99	-99	-99
110710	6	3	5	4894	-9	-99	-99	-99	5.10	0.65	0.74	5.37	-99	-99	-99
110711	6	4	1	350	-9	-99	-99	-99	1.64	0.33	0.09	4.91	-99	-99	-99
110711	6	4	2	913	-9	-99	-99	-99	4.88	0.38	0.52	5.26	-99	-99	-99
110711	6	4	3	1474	-9	-99	-99	-99	4.52	0.23	0.49	5.45	-99	-99	-99
110711	6	4	4	2327	-9	-99	-99	-99	4.88	0.62	0.59	5.18	-99	-99	-99
110711	6	4	5	4894	-9	-99	-99	-99	4.83	0.4	0.57	5.24	-99	-99	-99
110712	6	5	1	350	6	31.726	2215.9	2054.1	0.92	0.56	0.09	4.87	8.098	2.94	1.85
110712	6	5	2	913	6	31.670	2258.1	2127.8	4.50	0.25	0.46	5.25	8.010	2.51	1.58
110712	6	5	3	1474	6	31.721	2279.4	2173.3	4.12	0.25	0.38	5.43	7.939	2.19	1.38
110712	6	5	4	2327	6	31.693	2358.0	2187.6	4.55	0.32	0.49	5.07	8.101	3.15	1.98
110712	6	5	5	4894	6	31.666	2325.0	2306.4	4.68	0.41	0.51	5.13	7.655	1.22	0.77

TABLE 2. Data from Experiment #7.

FILE:	HDAT07-4.CAL														
DATE:	26JAN2013														
BY:	JPC														
# OF ROWS:	15														
# OF COLS:	16														
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DATESMPL	HTCEXP	EVENT	TANK	GASCO2	TEMP	SAL	ALKALIN	TCO2	NO3+2KG	NH4KG	PO4KG	SIKG	PHTTL	OMCA	OMAR
---	---	---	---	[CO2]	---	---	---	---	---	---	---	---	---	---	---
YYMMDD	#	#	#	PPM	DEGC	GM/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	PHUNIT	ULESS	ULESS
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
110722	7	1	1	350	-9	-99	-99	-99	3.37	1.21	0.58	2.90	-99	-99	-99
110722	7	1	5	4894	-9	-99	-99	-99	3.22	1.20	0.56	2.99	-99	-99	-99
110722	7	1	6	50000	-9	-99	-99	-99	3.23	1.14	0.6	2.99	-99	-99	-99
110723	7	2	1	350	6	31.771	2225.9	2071.5	3.25	1.12	0.57	2.99	8.076	2.83	1.78
110723	7	2	5	4894	6	31.777	2310.0 [a]	2341.1	3.26	1.14	0.55	2.95	7.487	0.84	0.53
110723	7	2	6	50000	6	31.785	2186.3	4169.7	3.37	1.54	0.60	3.01	6.095	0.03	0.02
110724	7	3	1	350	-9	-99	-99	-99	3.48	0.83	0.47	3.01	-99	-99	-99
110724	7	3	5	4894	-9	-99	-99	-99	3.48	1.12	0.47	2.99	-99	-99	-99
110724	7	3	6	50000	-9	-99	-99	-99	3.64	1.92	0.59	2.96	-99	-99	-99
110725	7	4	1	350	-9	-99	-99	-99	3.70	0.27	0.34	2.95	-99	-99	-99
110725	7	4	5	4894	-9	-99	-99	-99	3.71	0.19	0.33	2.90	-99	-99	-99
110725	7	4	6	50000	-9	-99	-99	-99	3.74	1.77	0.51	3.02	-99	-99	-99
110726	7	5	1	350	6	31.809	2234.0	2073.6	3.77	0.37	0.29	3.02	8.090	2.93	1.84
110726	7	5	5	4894	6	31.796	2320.4	2346.4	3.49	0.02	0.24	3.34	7.505	0.88	0.55
110726	7	5	6	50000	6	31.833	2196.4	4763.5	3.87	1.71	0.45	3.08	5.985	0.03	0.02

(a). Sample bottle broke. For pH calculation, the alkalinity value was estimated as 10.4 μmol/kg less than the value in tank 5 event 5.

TABLE 3. Data from Experiment #8.

FILE:	HDAT08-4.CAL														
DATE:	26JAN2013														
BY:	JPC														
# OF ROWS:	8														
# OF COLS:	16														
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
DATESMPL	HTCEXP	EVENT	TANK	GASCO2	TEMP	SAL	ALKALIN	TCO2	NO3+2KG	NH4KG	PO4KG	SIKG	PHTTL	OMCA	OMAR
---	---	---	---	[CO2]	---	---	---	---	---	---	---	---	---	---	---
YYMMDD	#	#	#	PPM	DEGC	GM/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	UMOL/KG	PHUNIT	ULESS	ULESS
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
110928	8	4	1	350	6	30.998	2181.1	2025.2	1.05	1.82	0.56	5.80	8.095	2.84	1.78
110928	8	4	2	2347	6	31.013	2233.0	2205.0	1.25	2.25	0.54	5.81	7.694	1.26	0.79
110928	8	4	3	4894	6	30.978	2235.7	2340.2	0.65	1.44	0.60	5.81	7.268	0.49	0.31
110928	8	4	4	8731	6	30.986	2220.1	2468.3	0.52	1.00	0.53	5.72	6.977	0.25	0.16
111002	8	12	1	350	6	31.007	2170.1	2031.1	0.01	0.13	0.31	5.89	8.052	2.60	1.63
111002	8	12	2	2347	6	31.015	2223.4	2187.3	0.44	0.25	0.29	5.88	7.723	1.34	0.84
111002	8	12	3	4894	6	30.982	2281.7	2260.9	0.42	0.43	0.31	5.88	7.670	1.22	0.76
111002	8	12	4	8731	6	30.997	2203.4	2488.9	0.18	0.10	0.30	5.86	6.920	0.22	0.14

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