A Tale of Two Tide Pools: Examining Carbonate Chemistry in the Intertidal

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ABSTRACT

Characterization of physical conditions in tide pools is quite common in the scientific literature, but there is a marked paucity of information regarding the carbonate chemistry of these ecosystems; the majority of studies focus on temperature, salinity, and dissolved oxygen measurements. This lack of information is especially worrisome in light of the predicted changes in carbonate chemistry (Ocean Acidification) likely to result from an increase in anthropogenic carbon dioxide in the atmosphere. In order to establish a baseline of comparison, we analyzed the carbonate chemistry of two tide pools at Dead Man's Cove on San Juan Island, WA. We found marked deviation in carbonate chemistry parameters in the tide pools from the nearby water of Haro Strait. We also attempted to elucidate the underlying causal factors of this deviation by adding biota to the tide pools, yet our results were inconclusive. We conclude that future work should focus on more extensive characterization of natural coastal and intertidal variation in carbonate chemistry parameters, as well as experimental design targeted at determining underlying causal factors of said variation in nature.

Keywords: carbonate chemistry, tide pool, Dead Man's Cove, Ocean Acidification

INTRODUCTION

Characterization of physical conditions in tide pools is quite common in the scientific literature, but the majority of the studies examine features such as dissolved oxygen, temperature, and salinity (Daniel & Boyden 1975; Goss-Cutard et al. 1978; Truchot & Duhamel-Jouve 1980). Considerably less work has examined the carbonate chemistry of tide pools; some papers specifically citing the difficulty of accurately assessing the different parameters of this system (Daniel and Boyden 1975). But the development of the research field of Ocean

Acidification has resulted in an increase in availability of the equipment necessary to accurately measure the different parameters, as well as an increased interest in the values of chemical parameters seen in nature. Much of what is known of carbonate chemistry in nature has focused on oceanographic research that rarely mirrors carbonate conditions found in near-shore waters (i.e. <100m from shore; Feely et al. 2008). To address this gap in knowledge, we took water samples from two tide pools in Dead Man's Cove on San Juan Island, WA in order to characterize the change in carbonate chemistry over the course of a tidal cycle. Our overarching goal was to establish the level of variation that would be seen over a tidal cycle in tide pools that were separated from the ocean by tidal action for at least six hours. A secondary question addressed in our study examines the possible biotic causative factors behind observed changes in carbonate chemistry. To answer this question, we added biota (*Ulva* sp. and *Mytilus trossolus*) to one of the tide pools in the hope of identifying whether photosynthesis or respiration was the source of variation naturally observed in a natural tide pool.

METHODS

Study Site

We selected two tide pools with similar tidal heights and relatively similar biota (to minimize natural background variation) in July of 2011 at Dead Man's Cove on San Juan Island, WA (N 48˚30.837, W123˚08.763, Fig. 1). Using a meter stick, we estimated the volume of each pool based on rough estimates of surface area and average depth. We then determined tidal height using stadia rods and a hand level. This was the main factor we used to select the pools; our goal was to control for variation between the pools as much as possible. Ultimately, we were forced to select one pool of estimated volume 0.4101m3 (reference pool) and another of 0.1031m3 (treatment pool). Though the difference between these pools is hardly ideal, it was the

closest we could get to finding two pools with similar biota and vertical height within the *Fucus* zone. We looked for pools in the *Fucus* zone such that the pools would be exposed for a large portion of the low tide and potentially increase the chance of detecting extreme variation in carbonate chemistry. We determined the abundance of macroalgal fauna as percent cover by species and described estimates of animal abundance using the following semi-quantitative scale: absent (none), present (1), few (< 10), abundant (< 50), and many (50+) (table 1).

Quantifying Chemical composition over a tidal cycle

We took water samples (500 ml) for analysis of carbonate chemistry from each pool at four time points during the tidal cycle: immediately after isolation from sound waters (time zero), 2 hours, 4 hours and right as sound waters were beginning to enter into the pools. We also took a sample of water from the cove adjacent to our tide pools (located > 100 m from the tide pools, within the cove, and at a depth of approximately 0.04 m) at the beginning and end of the tidal cycle in order to characterize the extent of physical and chemical fluctuations tide pool biota likely experience relative to the Strait. We collected samples in Schott Duran borosilicate glass bottles with a modified rubber stopper; we inserted tygon tubing into two holes in the top of the stopper to facilitate sample collection. The first hole had a piece of tubing long enough to reach above the level of the water to permit air displacement, and the second hole had tubing flush with the upper portion of the stopper extending to the bottom of the bottle. We used this design in order to minimize gas exchange and perturbation of the chemistry; when we placed the bottles under the surface of the water it flowed in smoothly with minimal bubbling. After sample collection, we removed the rubber stopper, poisoned the sample water with 100µL of saturated mercuric chloride and stored them according to the protocols described by Dickson et al. (2007) until analyzed. At time zero and every hour thereafter, temperature, salinity, and

dissolved oxygen (D.O.) were measured at the surface of each tide pool and in the Strait using a temperature probe and dissolved oxygen probe with an attached salinometer. On day two we did not measure D.O. due to equipment constraints. A TidBit temperature logger (OnSet) was also placed at the bottom of each tide pool at time zero and removed after the last sample was taken each day.

We measured dissolved inorganic carbon (DIC) and total alkalinity (TA) using a Licor infra –red analyzer and Agilent automatic titrator (model 34970). We measured pH using an Ocean Optics spectrophotometer with 10 cm pathlength optical cells following the methods of Dickson *et al*. (2007). As preliminary measurements of tide pool pH exhibited high pH values, we used two different dyes to measure pH: m-cresol purple can provide an accurate pH over the range of 7.4 to 8.2 (Dickson et al. 2007) whereas thymol blue as been reported to provide more accurate pH measurements for pH values > 8.0 (Zhang & Byrne 1996).

We mixed an 8×10^{-1} mol dm^{-3} solution of thymol blue in 10% ethanol, and 90% water under heat. To aid the dye in dissolution, we then added 600µL of NaOH while heating the dye. We calculated the pH of each sample on the spectrophotometer according to the procedure outlined by Zhang and Byrne (1996).

To check the accuracy of the measured pH values, we calculated pH at 25˚C in CO2Calc (Robbins et al. 2010) using the constants for Lueker *et al*. (2000), Dickson (1990b), and Wanninkhof (1992). These calculated values were closest to the *m*-cresol purple values. However, as *m*-cresol purple is not reported to be accurate above a pH of 8.2, we used the CO2Calc pH values in our analysis after they were corrected for sample temperature and reported on the total H^+ scale (mol kg^{-1} SW). Values for these various pH measurements can be found figure 2.

Certified reference materials (Andrew Dickson, Scripps Institute of Oceanography) were used to calibrate and verify accuracy of the machines.

Other parameters related to carbonate chemistry (i.e. aragonite saturation, partial pressure of CO_2) were calculated with CO2Calc using the constants mentioned above.

Tide Pool Manipulations

In order to better understand the relative contributions of photosynthesizing and respiring organisms, we added organisms to one of the tide pools on day two and day three (henceforward referred to as the 'treatment' pool). On day one, both the treatment and 'reference' pools were sampled without addition of any biota. On day two, we added *Ulva* sp. collected from tanks at Friday Harbor labs as well as the area surrounding our pools (approximate surface area of *Ulva* added was $1.04 m^2$) at time zero to the treatment pool and removed it after the last sample. The other, 'reference' pool remained unaltered on all days during our study. We collected mussels (*Mytilus trossulus*) used on day three from the dock at Argyle Creek prior to the start of the experiment (48°31'09.92"N, 123°00'47.68"W). We brought the mussels back to Friday Harbor labs where all encrusting biota were removed and they were housed at standard temperature, pressure, and salinity. We determined buoyant weight 24 hours prior to the addition of mussels to our treatment pool by suspending mussels in seawater from a bottom-loading balance (Davies 1989). On day three, we added 278.1 g (buoyant weight) of *M. trossulus* throughout the treatment pool at time zero and then removed them after the last sample.

Data Analysis

We analyzed all of our data using R (v. 2.13.00). In order to account for natural variability seen between our two pools, we normalized our data by dividing the difference in values (TA, DIC, etc) between our reference and treatment pool on day two and three by the

difference in values between the reference and treatment pool on day one (when no additions were made):

$$\frac{(\text{treatment pool} - \text{reference pool})_{\text{manipulation day}}}{(\text{treatment pool} - \text{reference pool})_{\text{non-manipulation day}}}$$

This transformation allowed us to see the magnitude of difference between the pools whilst controlling for natural environmental variation.

RESULTS

Though hardly identical in composition, the two pools we selected were quite similar in their biota (table 1 and 2) tidal height, and physical size (table 3). Despite this, our results still show notable environmental variation for all physical parameters measured (Figs. 3-8).

Temperature and D.O. increased over the course of sampling for all three days measured (table 3). D.O. eventually exceeded our ability to measure in both tide pools on both days measured. Salinity did not change more than 1.5‰ over the course of our observations (table 3). Temperature and D.O. were both higher in tide pools than in Haro Strait, but salinity did not deviate markedly from the strait.

Of the three main carbonate chemistry parameters measured in each water sample, both DIC and TA showed marked declines in both the treatment and reference pools over all three days of sampling (Fig. 3 and 4). Our normalization transformation indicates that the changes seen day to day were quite inconsistent (Fig. 5 and 6). pH, on the other hand, showed meaningful increase in both the treatment and reference pools over all three days of sampling (Fig. 7) and graphs normalized to the reference pool indicate that though the trend is very similar

over all three days (Fig. 8). All three parameters varied markedly from the water in Haro Strait by the completion of our sampling series (Fig. 3, 4, and 7, table 3).

The chemistry parameters calculated with CO2Calc (partial pressure of carbon dioxide [pCO2], aragonite saturation [Ω_{arg}], and bicarbonate [HCO_3^-]) show marked declines in both tide pools over all three days (table 3). By the end of our sampling on each day, each of these parameters varied markedly from the water of Haro Strait.

DISCUSSION

Though we had predicted that the chemistry of our tide pools would change over the course of a tidal cycle, the variation in several parameters we observed over the course of our observations and manipulations were much larger than we had expected. Though our experimental manipulations of biota in the treatment pool did not show a marked deviation from the trends seen in our reference pool, we believe these 'natural' changes we observed can be attributed to biotic rather an abiotic factors. Each tide pool characterized in our study had a significant amount of pre-existing biomass that we did not tamper with over the course of our study (table 1 and 2). We suspect that the amount of biomass already present in each pool was large enough relative to the biomass we added that any induced signal would be too small to observe beyond the natural variation already present in the pools. Despite this lack of an induced signal, the direction and magnitude of the chemical changes in each pool are indicative of the photosynthetic biota having a strong influence on the chemical parameters of tide pools.

Among the most notable of the changes we observed in our study was the dramatic decrease in DIC and TA (Fig. 3 and 4). We suspect that these decreases are due primarily to a decrease in [HCO_3^-], which decreases dramatically (table 3) over the course of a tidal cycle and

contributes to DIC and TA, as well as being the dominant carbon species at the pH values that we observed in our pools (Middelboe & Hansen 2007). We believe that the decline in [HCO_3^-] is best explained by the presence of so much algal biomass in each pool. Many algal species will readily use bicarbonate ions in photosynthesis as a source of DIC when carbon dioxide would otherwise be limiting (Murru & Sandgren 2004; Hurd et al. 2009). Despite the presence of many animals in our pools that would be respiring carbon dioxide throughout the tidal cycle, the partial pressure of carbon dioxide (pCO_2) decreases substantially during our observations, which would theoretically limit the photosynthesis of the algal species present if they were incapable of using bicarbonate ions (table 3). Yet the D.O. of each pool exceeded our ability to measure on every day measured, which indicates that the algae in the pool were photosynthesizing actively throughout the tidal cycle and the animals present were not respiring enough to limit the oxygen present in the pool (table 3). This suggests to us that the algae were actively photosynthesizing throughout the tidal cycle through the uptake of bicarbonate ions, and thus had a very large impact on the carbonate chemistry of each tide pool.

Another parameter that suggests that algae is having a dominant effect on the carbonate chemistry of our tide pool is in the increase in pH observed in each tide pool on each day of our study (Fig. 7). This may initially seem counter-intuitive in light of the decline of bicarbonate (a basic species) discussed previously, but an increase in pH of seawater surrounding algal species is well described in literature pertaining to algal physiology (Murru & Sandgren 2004). This is as a result of the fact that uptake of any form of DIC by algal cells results in production of hydroxyl ions, which leads to an increase in pH (Murru & Sandgren 2004). Algal physiologists will make use of this phenomenon to measure a specific alga's ability to uptake DIC in closed experimental conditions. Obviously in our system there are too many other possible factors contributing to the

change in pH, but it is reasonable (based upon our biomass estimates and observed changes) to assume that this reaction is occurring in our tide pools. Furthermore, if the dominant driver of chemical change in the water of these tide pools were animals, the pH should theoretically decline as more CO_2 (a weak acid) was added to the water through respiration, yet as we have seen, the pCO_2 of the tide pool water declined dramatically over the tidal cycle (table 3). This increase in pH (possibly coupled with the decrease in pCO_2) is likely also a dominant driver of the dramatic rise in Ω_{arg} seen over the course of the tidal cycle (table 3).

In conclusion, the carbonate chemistry of the two tide pools examined in this study varied dramatically from the surrounding water in the Haro Strait. Though our experiment was unable to definitively demonstrate if the source of this variation is biotic or abiotic, our observations of both our treatment and reference tide pools suggest that the main driver of these changes is photosynthetic algae. The magnitude of the changes observed also have implications for future research into carbonate chemistry, including future studies focused on ocean acidification. This study definitively shows that tide pool organisms are subjected to a dramatic range of extreme yet short-term variations in water chemistry, which suggests that they may be differently adapted to changes in carbonate chemistry than other, more subtidal organisms. Care must be taken when selecting focal organisms for future ocean acidification research; knowledge of natural variation in water chemistry focal organisms are adapted to is critical to selection of proper experimental conditions.

Finally, this primarily descriptive study has shed light on the need for more extensive characterization of tide pool chemistry. Future work should have a twofold focus; characterization of variation in carbonate chemistry due to weather, and experimental manipulation of biomass. The variability in our data between days suggests that there could be

significant differences in chemical parameters of tide pools throughout the year due to seasonal weather patterns, but only extensive, long term studies would be able to definitively demonstrate these changes and give a better picture of the sort of variability these tide pool organisms have to deal with year round. Additionally, there is likely a great deal of variation between tide pools with different biota: experimental manipulation including artificial tide pools, defoliation of existing tide pools, or more extensive biomass additions to tide pools will definitively demonstrate which component of tide pool biota is the major driver of changes in carbonate chemistry in nature.

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So long...and thanks for all the fish.

TABLES

Table 1: record of abundance of animals in tide pools (Few = <10, Many = 10-50, Abundant = >50).

Tidepool	Present	Few	Many	Abundant
Reference		chiton spp., *Semibalanus cariosus*, amphipod spp., *Calliostoma spp.*, *Littorina sitkana*, *Nucella spp.*		Medium and large *Pagarus spp.*, *Lottia spp.* (at least 2 different kinds)
Treatment	nemertean worm, *Katharina tunicata*	*Hemigrapsus nudus*	*Littorina sitkana*, small *Pagarus* spp, *Spirorbidae* spp., limpet spp., *Littorina* complex	*Oligocottus maculosus*, *Nucella*

Table 2: Algal composition of the two tide pools.

Tidepool	Species	Percent Cover
Treatment	Crustose coralline	40%
	Corallina vancouveriensis and *Bossiella plumosa.*	35%
	Ulva sp.	15%
	Polysiphonia sp.	<1%
	Porphyra sp.	<1%
	Fucus distichus i.	<1%
	Saccharina sessilis	<1%
	Hallosaccion glandiforme	<1%
	Microcladia coulteri	<1%
	Odonthalia floccosa	<1%
Reference	Crustose coralline	10%
	Corallina vancouveriensis and *Bossiella plumosa*	55%
	Fucus distichus. (loose)	5%
	Odonthalia floccosa	30%
	Prionitis lanceolata	<5%
	Leathesia marina	<5%
	Soranthera ulvoidea	<1%

Table 3: measured parameters of all samples taken over the course of our study. For D.O., '>200%' represents occasions when the level of oxygen in the water sample exceeded our meter's ability to accurately read, and 'n/a' is from day 2, when we did not have access to the meter to take measurements in the field.

SampleID	Day	Location	D.O. (% Saturation)	Temperature (°C)	Salinity (‰)	pH	TA (µmol/kg)	DIC (µmol/kg)	pCO2 (µatm)	HCO3 (µmol/kg)	Ωarg
TP001	1	Reference	43.2%	10.25	30.1	7.187	2116.5	2171.5	3451.9	2042.5	0.392
TP002	1	Treatment	42.8%	10.10	30.8	7.260	2096.0	2125.4	2861.5	2008.2	0.470
TP003	1	Haro Strait	61.5%	10.17	30.4	7.528	2128.8	2077.2	1520.1	1969.0	0.913
TP004	1	Reference	95.7%	11.32	30.0	7.490	2065.4	2028.2	1620.4	1924.7	0.801
TP005	1	Treatment	125.9%	10.98	30.3	7.700	2055.0	1951.5	951.1	1835.4	1.292
TP006	1	Reference	224.4%	15.40	31.3	8.219	1802.4	1482.3	197.9	1268.0	3.202
TP007	1	Treatment	>200%	17.66	31.5	8.444	1819.8	1355.5	98.4	1055.6	4.641
TP008	1	Treatment	>200%	18.51	30.8	8.558	1753.3	1237.0	65.4	910.2	5.127
TP009	1	Reference	>200%	17.96	31.3	8.482	1669.9	1216.1	79.6	933.7	4.388
TP010	1	Haro Strait	84.8%	12.25	30.1	7.648	2078.9	1994.6	1099.2	1883.5	1.156
TP011	2	Reference	n/a	10.20	30.0	7.261	2089.5	2121.3	2853.9	2004.4	0.458
TP012	2	Treatment	n/a	10.15	30.1	7.433	2081.8	2060.5	1879.0	1955.7	0.704
TP013	2	Haro Strait	n/a	10.13	29.6	7.478	2155.0	2123.1	1746.0	2015.7	0.793
TP014	2	Reference	n/a	11.39	30.1	7.616	1981.4	1908.3	1134.8	1804.5	1.025
TP015	2	Treatment	n/a	11.24	30.2	7.972	2045.5	1838.1	463.8	1674.9	2.238
TP016	2	Reference	n/a	13.73	30.1	8.127	1833.1	1570.3	268.9	1386.0	2.689
TP017	2	Treatment	n/a	13.71	30.2	8.436	1922.8	1466.8	110.3	1161.3	4.664
TP018	2	Treatment	n/a	17.41	30.2	8.811	1740.2	1078.0	26.7	667.5	6.511
TP019	2	Reference	n/a	18.12	30.1	8.577	1577.7	1099.4	55.5	808.1	4.575
TP020	2	Haro Strait	n/a	11.27	30.1	7.611	2094.2	2021.2	1215.7	1912.0	1.066
TP021	3	Reference	63.4%	10.40	30.4	7.417	2061.3	2046.3	1926.3	1942.4	0.662
TP022	3	Treatment	77.6%	10.27	30.8	7.533	2078.7	2027.9	1458.0	1922.7	0.889
TP023	3	Haro Strait	77.4%	10.25	30.8	7.525	2132.2	2083.7	1525.1	1976.1	0.892

TP024	3	Reference	87.2%	11.00	30.8	7.468	2015.5	1984.4	1656.6	1883.4	0.743
TP025	3	Treatment	111.3%	11.10	31.0	7.630	2030.5	1950.0	1114.0	1841.9	1.094
TP026	3	Reference	140.8%	12.00	30.7	7.836	1893.2	1747.8	612.8	1622.7	1.592
TP027	3	Treatment	196.3%	12.20	30.9	7.916	1956.2	1774.2	510.9	1628.7	1.952
TP028	3	Treatment	>200%	13.80	30.8	8.290	1836.4	1479.1	164.4	1240.2	3.584
TP029	3	Reference	>200%	14.70	30.6	8.179	1691.8	1414.5	210.8	1230.1	2.729
TP030	3	Haro Strait	73.4%	11.30	30.8	7.549	2085.5	2030.7	1405.1	1924.9	0.926

FIGURES

Figure 1: Dead Man's cove, San Juan Island, WA (N 48˚30.837, W123˚08.763). Sampling sites are marked by yellow 'x'.

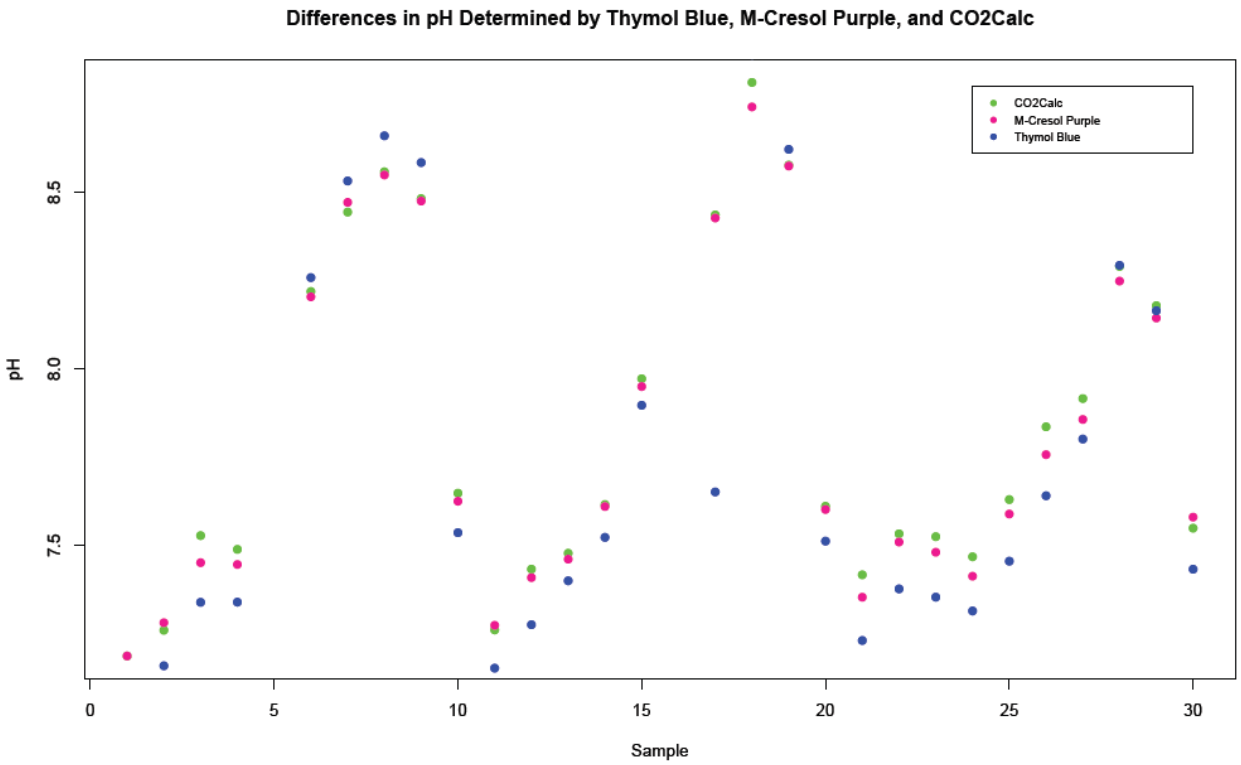
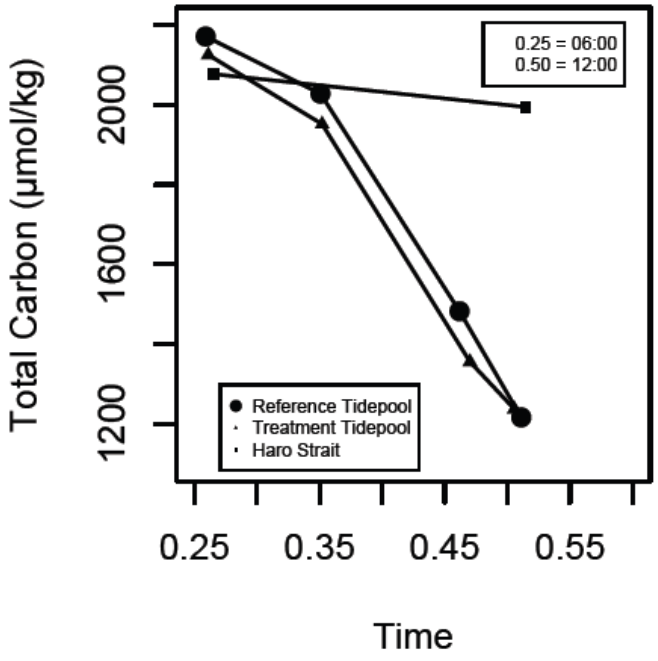
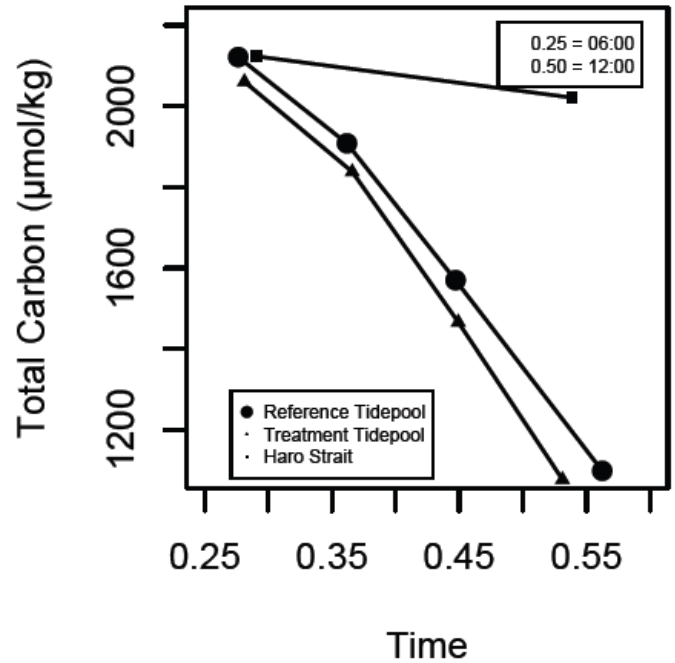


Figure 2: differences between calculated (green), measured with M-Cresol Purple (pink), and measured with Thymol blue (blue) values for pH from tide pools and Haro Strait.

Change in Carbon Content throughout Day 1



Change in Carbon Content throughout Day 2



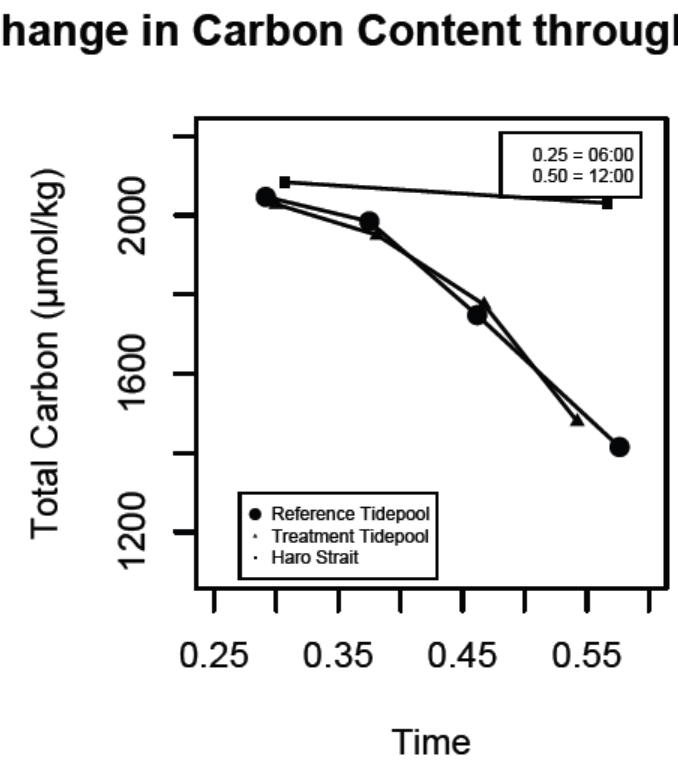
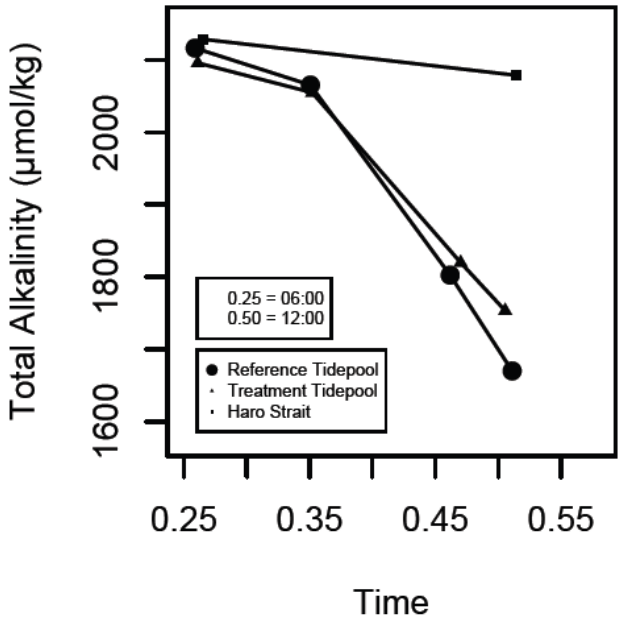
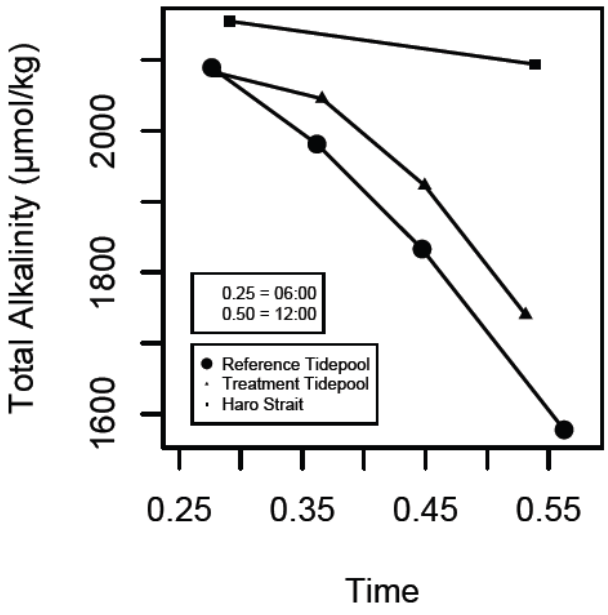


Figure 3: dissolved inorganic carbon (DIC) as a function of time. Each graph represents samples from one day. Each line on the graph represents the water samples taken from Haro Strait, the reference tide pool, or the treatment tide pool. Day 1 no experimental manipulations were carried out; day 2 *Ulva* was added to the treatment tide pool; day 3 *Mytilus trossulus* was added to the treatment tide pool.

Change in Total Alkalinity throughout Day 1



Change in Total Alkalinity throughout Day 2



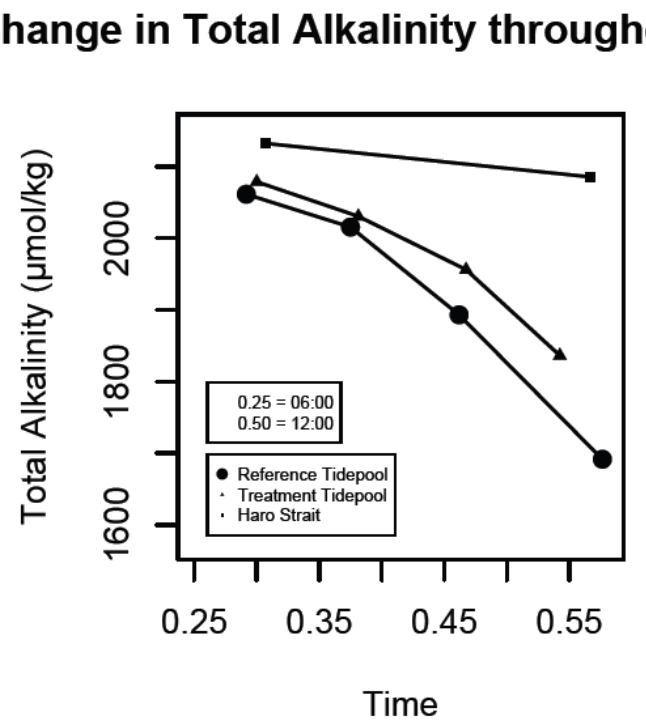


Figure 4: total alkalinity (TA) as a function of time. Each graph represents samples from one day. Each line on the graph represents the water samples taken from Haro Strait, the reference tide pool, or the treatment tide pool. Day 1 no experimental manipulations were carried out; day 2 *Ulva* was added to the treatment tide pool; day 3 *Mytilus trossulus* was added to the treatment tide pool.

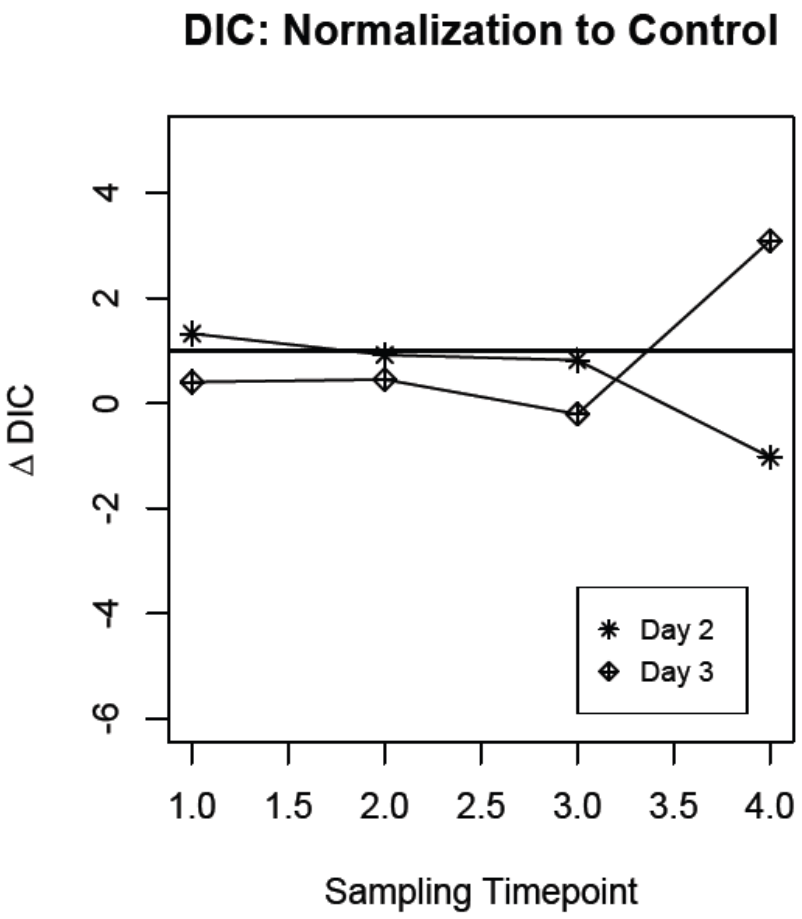


Figure 5: changes in dissolved inorganic carbon (DIC) from experimental manipulations normalized to changes seen on day one of our study. The horizontal line at +1 indicates a situation where the difference between the treatment and reference pool on the experimental day is the same as the difference between the treatment and reference pool on the first day of the study (i.e. no experimental manipulation).

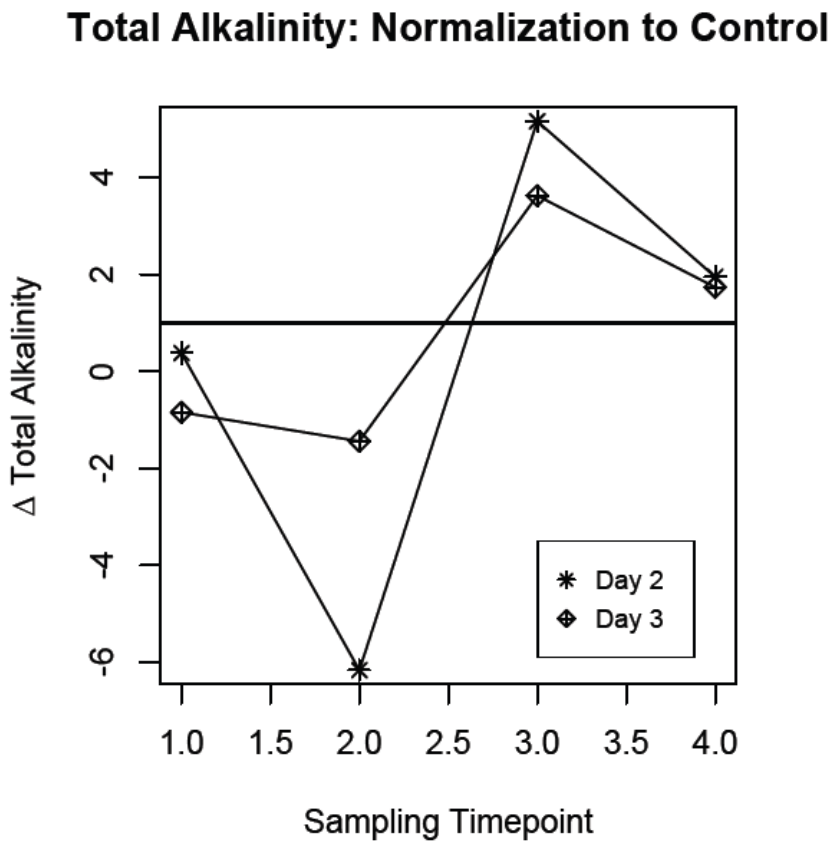
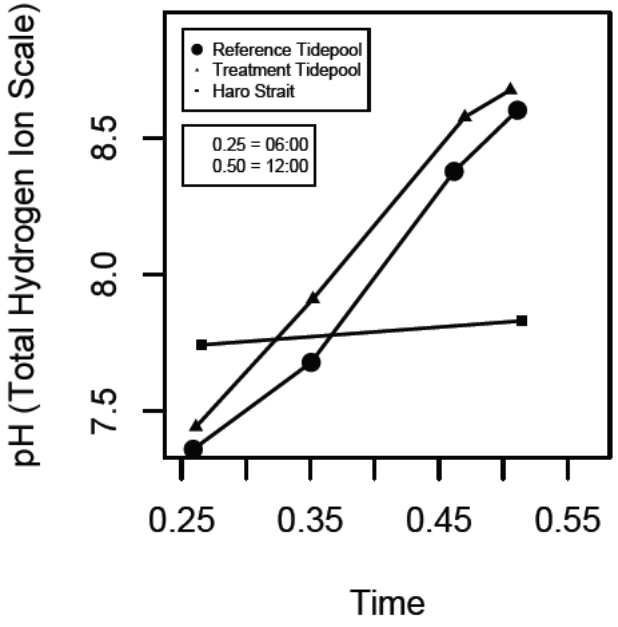
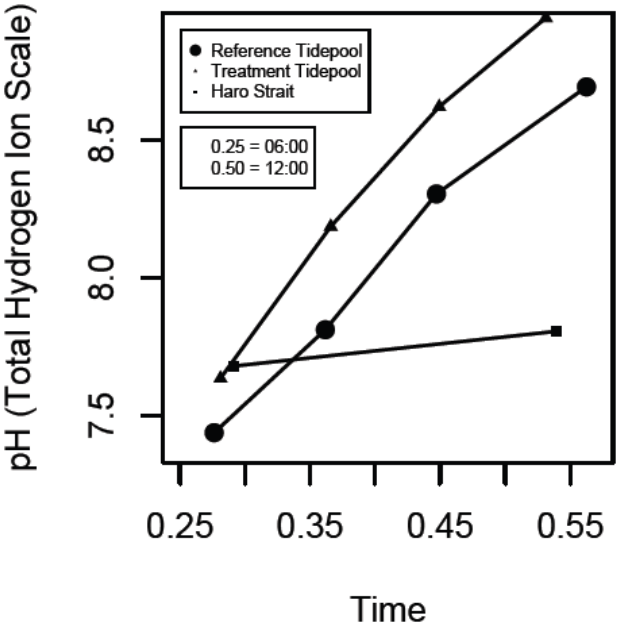


Figure 6: changes in total alkalinity (TA) from experimental manipulations normalized to changes seen on day one of our study. The horizontal line at +1 indicates a situation where the difference between the treatment and reference pool on the experimental day is the same as the difference between the treatment and reference pool on the first day of the study (i.e. no experimental manipulation).

Change in pH throughout Day 1



Change in pH throughout Day 2



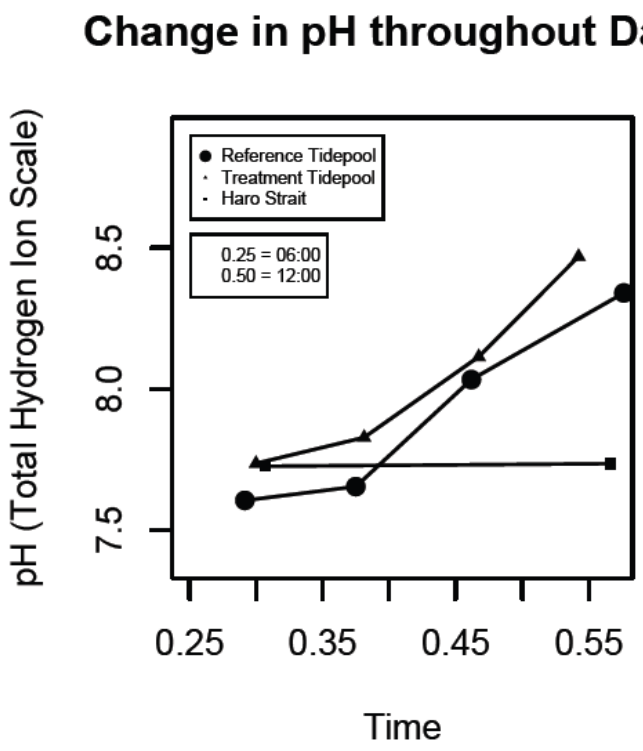


Figure 7: pH as a function of time. Each graph represents samples from one day. Each line on the graph represents the water samples taken from Haro Strait, the reference tide pool, or the treatment tide pool. Day 1 no experimental manipulations were carried out; day 2 *Ulva* was added to the treatment tide pool; day 3 *Mytilus trossulus* was added to the treatment tide pool.

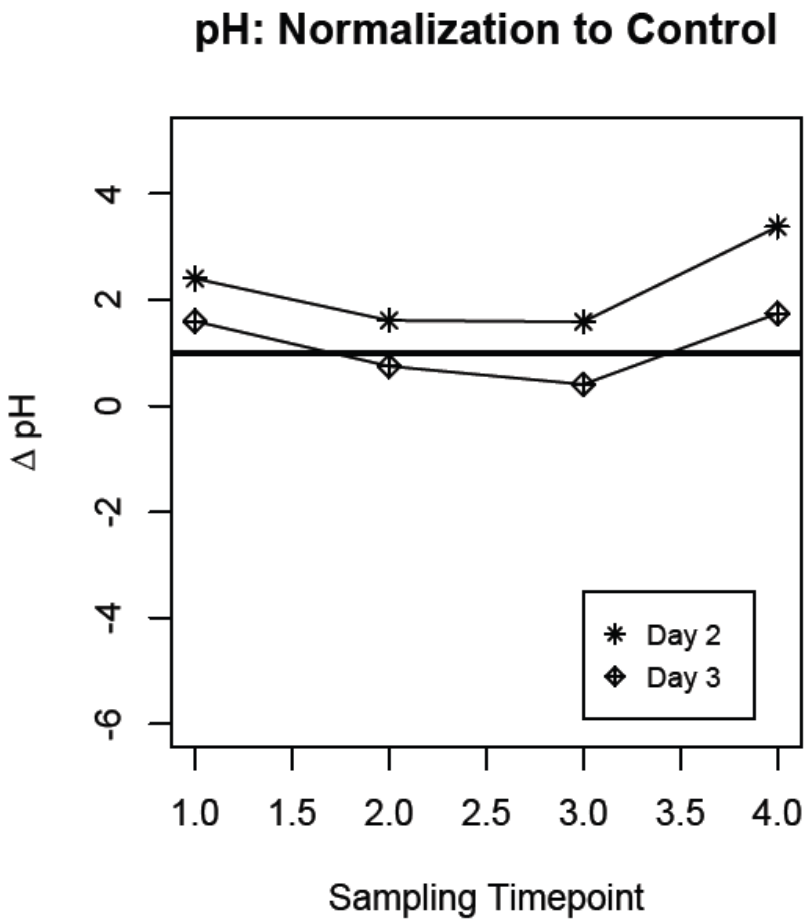


Figure 8: changes in pH from experimental manipulations normalized to changes seen on day one of our study. The horizontal line at +1 indicates a situation where the difference between the treatment and reference pool on the experimental day is the same as the difference between the treatment and reference pool on the first day of the study (i.e. no experimental manipulation).

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