Investigation of the carbonate chemistry of two tide pools on San Juan Island

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Abstract

Open ocean observations have shown that increasing levels of anthropogenically-derived atmospheric CO_2 are causing acidification of the world's oceans. Yet little is known about coastal acidification and very few studies have characterized the carbonate chemistry of coastal zones or intertidal rock pools where many calcifying organisms exist. In addition to various physical parameters (temperature, dissolved oxygen (D.O.), and salinity), we characterized the carbonate chemistry by taking measurements of pH, total alkalinity, and dissolved inorganic carbon (DIC) of two tide pools (0.41 m^3 and 0.10 m^3) located at Dead Man's Cove on San Juan Island, Washington, to determine the extent of variation over the course of one tidal cycle and compared those results to local water samples taken from the Strait of Juan de Fuca. Both tidal pools exhibited extreme values in carbonate chemistry over the course of the day (for the 0.41 m^3 tide pool: 7.36 – 8.60 pH, 2116.53 – 1669.86 μmol kg^{-1} total alkalinity, 2171.5 – 1216.1 μmol kg^{-1} DIC, and 2088.494 – 57.448 μatm pCO_2) and calcite and aragonite saturation states (0.623-6.84 Ω Ca, 0.392-4.388 Ω Ar). On separate, consecutive days, the alga *Ulva* sp. and the mussle *Mytilus trossulus* were added to the smaller of the tide pools to observe the potential impact of photosynthetic versus respiring biomass on the carbonate chemistry. Adding algae resulted in an expected increase in pH, a decrease in DIC, and an increase in Ω Ca and Ω Ar. Total alkalinity decreased less than expected; perhaps due to the shading of coralline algae by *Ulva* which may have prevented coralline calcification. pCO_2 decreased less than expected on day 2 relative to day 1 in the treatment pool and may have been due to changes in weather relative to the first day. Addition of mussels resulted in an expected increase in DIC, an increase in pCO2 and reductions in Ω Ca and Ω Ar. D.O. did not increase and pH did not decrease as much as expected on day 3 in the

treatment pool and these unexpected findings may be due to the fact that mussels were not allowed to acclimate. Excretion of ammonium or addition of organic acids from added mussels may have offset a reduction in total alkalinity. This study demonstrates that many intertidal species are experiencing far greater fluctuations in carbonate chemistry than expected and has significant implications for ocean acidification experiments where intertidal species are used.

INTRODUCTION

It has been predicted that with the continued anthropogenic input of carbon dioxide (CO_2) the pH of surface ocean waters could decrease by as much as 0.3 – 0.5 units by the year 2100 and as much as 0.7 units by 2250 (Caldeira and Wickett 2003). The carbonate chemistry (CO_2, HCO_3^-, CO_2^{3-}) of surface waters of the world's open oceans has been well-characterized yet few studies have examined the chemistry of coastal zones, especially the intertidal.

Biological processes such as photosynthesis and respiration affect carbonate chemistry; photosynthesis removes CO_2 from the surrounding water whereas respiration adds it. Photosynthesis of submerged macroalgae increases the pH of the surrounding water (e.g., Pearson et al 1998) while decreasing DIC (mainly through uptake of CO_2 and HCO_3^-). Respiration of marine organisms decreases the pH but increases DIC due to the increase in CO_2. Addition or removal of CO_2 does not impact total alkalinity; for every molecule of CO_2 added or removed, a corresponding molecule of HCO_3^- and H^+ are also added/removed (see Wolf-Gladrow et al 2007 for a discussion). Yet organisms can

reduce total alkalinity through calcification and excretion of nutrients (e.g., nitrate) or increase total alkalinity by incorporation of phosphate (Wolf-Gladrow et al 2007).

Little is known about the carbonate chemistry of the intertidal zone around San Juan Island. The objective of this study was to characterize the carbonate chemistry of two tide pools over the course of a tidal cycle and then observe how the addition of photosynthetic biomass versus respiring biomass affected the carbonate chemistry. We hypothesized that once isolated (from the waters of the Haro Strait), both tide pools would experience an increase in D.O., a decrease in pCO2, a decrease in DIC, an increase in pH, and a decrease in total alkalinity due to the photosynthetic processes taking place during the day. We hypothesized that the addition of *Ulva* sp. would further exacerbate these conditions whereas the addition of *Mytilus trossulus* would work to counteract the photosynthetic processes normally taking place within the pool and therefore most of the conditions mentioned above (D.O. increase, pCO2 decrease, DIC decrease, pH increase, and a decrease in total alkalinity) would occur to lesser extent than on the days where no additional respiring organisms were added.

METHODS

Study Site

Two rock tidal pools with similar tidal heights and relatively similar biota were selected in July of 2011 at Dead Man's Cove on San Juan Island, WA (N 48˚30.837, W123˚08.763, figure 1). Using a meter stick, volume was based on rough estimates of surface area and average depth. Tidal height was determined by using a stadia rod and a hand level and was the main factor used to select the pools in order to control for

variation as much as possible. With respect to tidal height, we looked for pools in the *Fucus* zone; thus the pools would be exposed for a large portion of the low tide and potentially increase the chance of detecting extreme variation. Abundance of macroalgal fauna was determined as percent cover by species and estimates of animal abundance were obtained by using the semi-quantitative scale: present/absent (none/1), few (< 10), abundant (< 50), and many (50+) (table 1).

Quantifying Chemical composition over a tidal cycle

Samples (500 ml) for carbonate chemistry were obtained from each pool at four time points during the tidal cycle: shortly after isolation from Haro Strait waters (time zero), 2 hours, 4 hours and shortly before Strait water began to enter the pools. A sample of water from the Haro Strait (located > 500 m from the tide pools within the cove and at a depth of approximately 0.04 m) was also taken close to the beginning and end of the tidal cycle in order to characterize the extent of physical and chemical fluctuations tidal pool biota likely experience relative to the Strait. Samples were collected using a modified version (a rubber stopper with and air hole and tygon tubing running from the top of the stopper to the bottom of a Schott Duran borosilicate glass bottle) of the dissolved oxygen collecting device described in Daniel and Boyden (1975) in order to minimize gas exchange and perturbation of the chemistry. Samples were poisoned with mercuric chloride at the time of collection and stored according to the protocols described by Dickson et al. (2007) until analyzed. At time zero and every hour thereafter, temperature, salinity, and D.O. were measured at the surface of each tide pool and in the Strait using a Hart Scientific temperature probe (model 1522), a Hach sension 5 salinometer and a D.O. probe. On day two D.O. was not measured. A temperature data 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.

DIC and total alkalinity were measured using a Licor infra –red analyzer and Agilent automatic titrator (model 34970). pH was measured 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, two different dyes were utilized 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 and Byrne 1996).

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

To check the accuracy of the measured pH values, pH was calculated at 25˚C in CO2Calc using the constants for Lueker *et al.* (2000), Dickson (1990), 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, the CO2Calc pH values were used in the 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 in figure 2.

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

Tide pool Manipulations

In order to better understand the relative contributions of photosynthesizing and respiring organisms, organisms were added to one of the tide pools (referred to as treatment) on day 2 and day 3. This addition was performed on the smaller tide pool of the two in order to increase the likelihood of detecting a signal. On day one, both tide pools were sampled without addition of any biota. On day two, 1.04 m^2 of *Ulva* sp. collected from nearby tide pools was added at time zero to the small tide pool and removed after the last sample. In order to determine surface area, circular discs (n=11) of *Ulva* sp. were cut with a cork borer to obtain a standard area to dry weight ratio. These discs were dried in an oven (60^{o}C, 24hrs) and dry weights were obtained with an analytical balance. The remainder of *Ulva* sp. used in this experiment was also dried in an oven (60^{o}C, 72hrs). The standard area to dry weight ratio of the discs was then applied to the total *Ulva sp.* added to the pool to obtain the total area.

Mussels used for day three were collected from the dock at Argyle Creek six days prior to the start of the experiment. *M. trossulus* were brought back to Friday Harbor labs where all encrusting biota were removed. Buoyant weight was determined 24 hours prior to addition of mussels by suspending mussels in seawater from a bottom-loading balance (Davies 1989). Mussels were maintained in a seawater table until addition. On day three, 278.1 g (buoyant weight) of *M. trossulus* were added throughout the small tide pool at time zero and then removed after the last sample.

Analysis

In order to better understand the impact of photosynthesis and respiration to the carbonate chemistry of tide pools, the following equation was used to potentially remove

any ‘noise’ that might result from differences between days. This was done using the following equation:

$$\frac{(\text{Treatment} - \text{Reference})\text{ex}}{(\text{Treatment} - \text{Reference})\text{con}}$$

where ex was the day that the tide pool referred to as ‘treatment’ was experimentally manipulated (either by algae addition or mussel addition) and con was the day that the tide pools were observed without manipulation (e.g., day 1). Therefore treatment refers to the value observed in the treatment pool and reference refers to the value of the reference pool (e.g., the tide pool that was not manipulated). The bottom value remained the same regardless of the manipulated day, thus any true change detected on a particular experimental day could be more readily observed from the data. Therefore, if there were no differences between days then the value of this ratio would be 1.0.

Figure 1: Location of sampling sites in Dead Man's Cove, San Juan Island, WA (N 48˚30.837, W123˚08.763). An X marks an approximate location of the two tide pools and the location where we collected a sample from Haro Strait.

RESULTS

One pool (referred to as 'reference') was significantly larger in volume (0.41 m^3 versus 0.10 m^3) than the other pool (referred to as 'treatment'). The biota in each tide pool was considerably different (table 1). On the first day of observations (no manipulations done to tide pools) all parameters varied significantly over the course of the day both within and between tide pools (table 2, fig 2 - 8). Salinity and D.O. increased the most on day 1 (fig 2). Solar radiation (taken from the Friday Harbor Labs station) was highest on day 1 and decreased each consecutive day (fig 9). Temperature from the data loggers also followed the same trend but temperatures in the smaller pool (treatment pool) were higher (fig 3). For the reference tide pool, the pH was similar on

Table 1a: Mobile abundance in each tide pool

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

Table 1b: Algal composition of the two tide pools based on percent cover.

Tidepool	Species	Percent Cover
Treatment	Crustose coralline	40%
	Corallina vancouveriensis and *Corallina bossiella*	35%
	Ulva spp.	15%
	Siphonous	<1%
	Porphyra	<1%
	Fucus spp.	<1%
	Saccharina	<1%
	Hallosaccion	<1%
	Microcladia	<1%
	Odonthalia	<1%
Reference	Crustose coralline	10%
	Corallina vancouveriensis and *Corallina bossiella*	55%
	Fucus spp. (loose)	5%
	Odonthalia	30%
	Prionitis	<5%
	Leathesia	<5%
	Soranthera	<1%

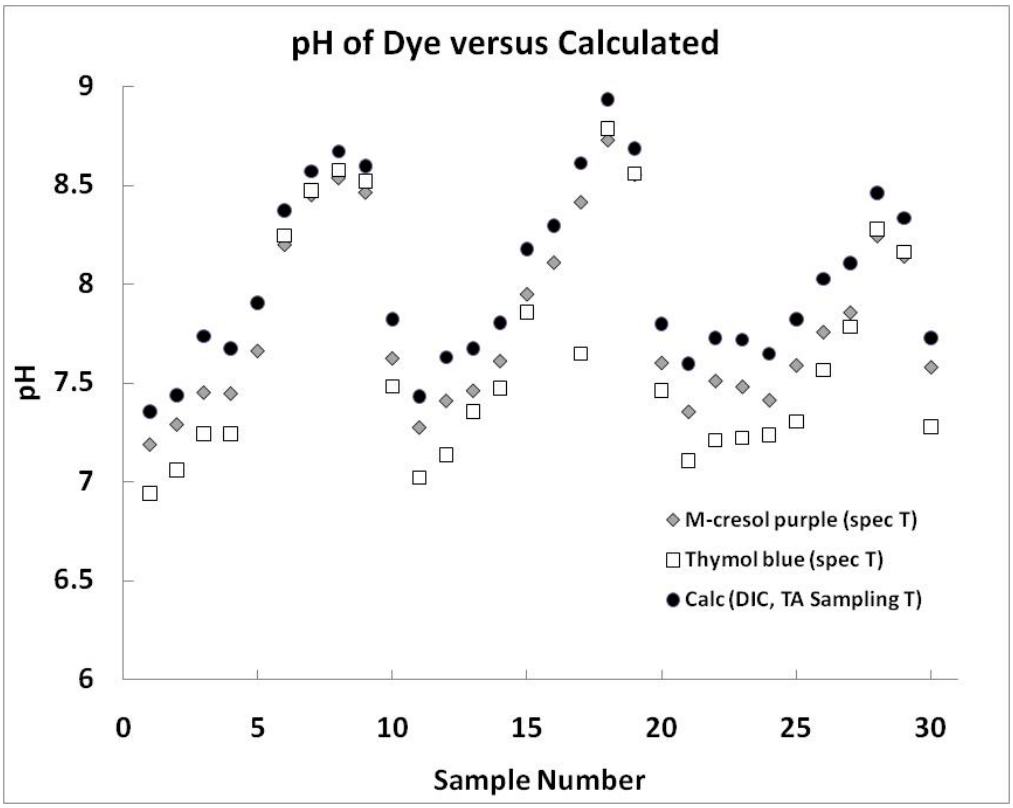


Figure 2. Various pH values measured. Dye values were calculated using temperatures of the samples in the spectrophotometer (spec T) whereas calculated values were given for temperatures recorded in the field (sampling T). Calculated values are what are presented in graphs below.

days 1 and 2 but less on day 3 whereas in the treatment pool, pH was greater on the algal addition day (day 2) and less on the mussel addition day (day 3) relative to the control day (day 1; fig 4).). Yet relative to the samples taken from the Haro Strait, pH increased dramatically in both tide pools over the course of the day with the largest ranges being seen on day 2 (1.26 and 1.31 pH units in the reference and treatment pools respectively versus 0.13 in the Strait). Total alkalinity of the tide pools also decreased much more greatly than in the Strait (60.89 μmol kg^{-1} on day 2) with the largest decreases observed

Table 2. Measured values of temperature (t), salinity (S), dissolved oxygen (%), pH (calculated from total alkalinity (TA) and DIC), total alkalinity (TA), dissolved inorganic carbon (DIC), pCO_2 and calcite and aragonite saturation states (ΩCa and ΩAr respectively) at two-hour intervals over the course of a tidal cycle for days 1, 2 (algae addition) and 3 (mussel addition) for both tide pools (reference and manipulated treatment pool). Also shown are samples taken at the beginning and end of the sampling period from the Haro Strait.

Time	t (^{0}C)	S (‰)	D.O. (%)	pH	TA (µmol kg$^{-1)}$	DIC (µmol kg-1)	pCO_2 (µatm)	Ωca	Ωar
Reference Pool (Day 1)									
6:13	10.25	30.1	43.20	7.36	2116.53	2171.5	2088.494	0.623	0.392
8:25	11.322	30.0	95.70	7.67	2065.41	2028.2	956.176	1.27	0.801
11:05	15.543	31.3	na	8.37	1802.41	1482.3	128.792	5.021	3.202
12:16	17.960	31.3	na	8.60	1669.86	1216.1	57.448	6.84	4.388
Strait (Day 1)									
6:22	10.25	30.1	43.20	7.74	2128.81	2077.2	835.838	1.45	0.913
12:20	12.246	31.1	84.80	7.83	2078.88	1994.6	663.272	1.831	1.156
Reference Pool (Day 2)									
6:38	10.201	30	na	7.43	2089.51	2121.319	1713.98	0.729	0.458
8:41	11.390	30.1	na	7.81	1981.39	1908.334	659.179	1.626	1.025
10:44	13.732	30.1	na	8.30	1833.05	1570.315	165.676	4.246	2.689
13:30	18.122	30.1	na	8.69	1577.71	1099.405	40.694	7.154	4.575
Strait (Day 2)									
6:59	10.130	29.6	na	7.67	2154.99	2123.102	991.282	1.263	0.793
12:57	11.273	30.1	na	7.80	2094.1	2021.228	707.65	1.691	1.066
Reference Pool (Day 3)									
7:00	10.395	30.4	63.40	7.60	2061.31	2046.3	1133.056	1.052	0.662
9:00	na	30.8	87.20	7.65	2015.5	1984.4	984.36	1.178	0.743
11:05	na	30.7	140.80	8.03	1893.15	1747.8	356.957	2.518	1.592
13:50	14.7	30.6	200.00	8.34	1691.79	1414.5	135.886	4.294	2.729
Strait (Day 3)									
7:22	10.25	30.8	77.40	7.72	2132.2	2083.7	870.572	1.416	0.892
13:36	11.3	30.8	73.40	7.73	2085.47	2030.7	837.139	1.466	0.926
Treatment Pool (Day 1)									
6:16	10.10	30.8	42.80	7.44	2095.97	2125.4	1695.185	0.745	0.47
8:27	10.98	30.3	125.90	7.91	2055	1951.5	532.159	2.05	1.292
11:17	17.658	31.5	> 200	8.57	1819.8	1355.5	68.688	7.236	4.641
11:56	18.506	30.8	> 200	8.67	1753.29	1237	47.475	7.994	5.127
Treatment Pool (Day 2)									
6:45	10.149	30.1	na	7.63	2081.83	2060.516	1063.653	1.118	0.704
8:47	11.238	30.2	na	8.18	2045.46	1838.12	258.29	3.55	2.238
10:47	13.707	30.2	na	8.61	1922.78	1466.837	66.972	7.362	4.664
12:45	17.412	30.2	na	8.94	1740.23	1077.955	18.662	10.2	6.511
Treatment Pool (Day 3)									
7:12	10.27	30.8	77.60	7.73	2078.66	2027.9	828.506	1.409	0.889
9:09	na	31.0	111.30	7.82	2030.51	1950	645.006	1.731	1.094
11:13	12.2	30.9	196.30	8.11	1954.01	1774.2	297.122	3.084	1.952
13:01	13.8	30.8	na	8.46	1836.44	1476.1	101.05	5.646	3.584

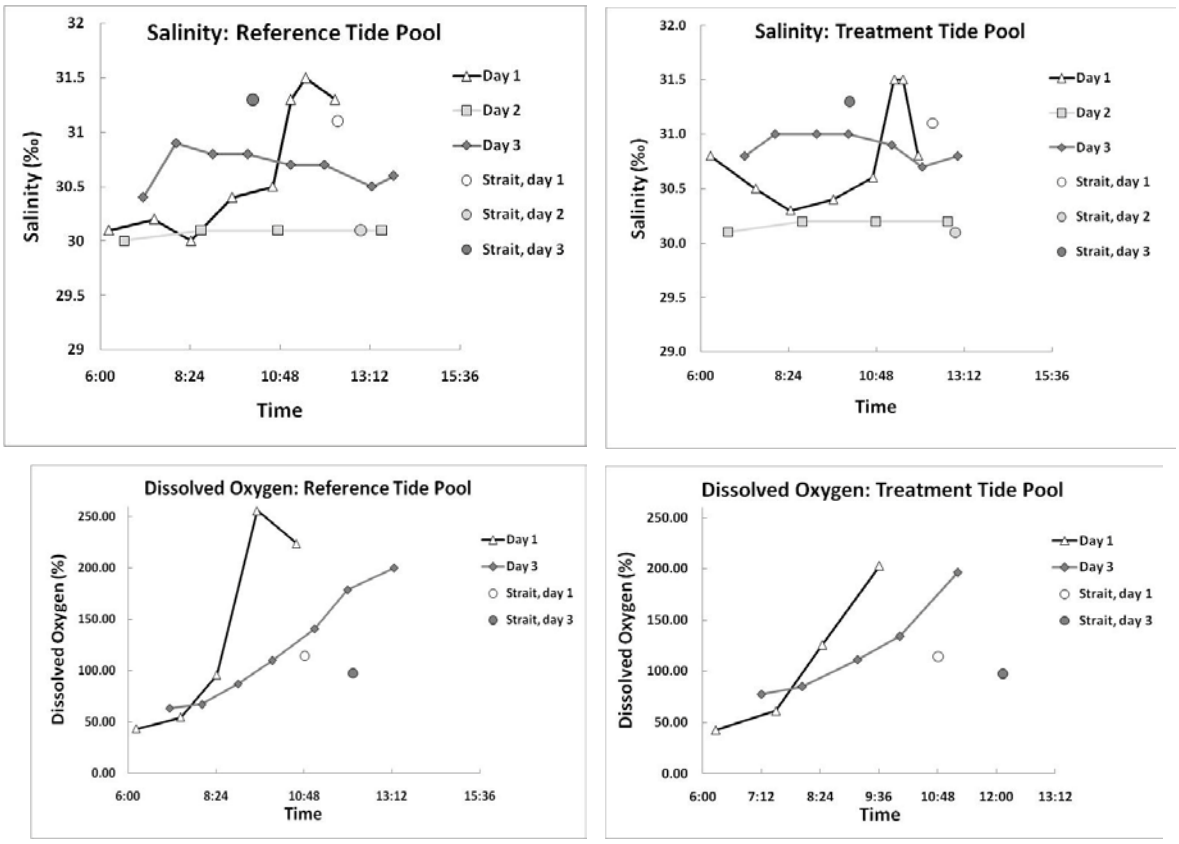


Figure 2. Salinity and dissolved oxygen of reference and treatment tide pools on all 3 days; D.O. was not measured on day 2. Algal addition was on day 2, mussel addition was on day 3.

on day 1 for the treatment pool (342.68 μmol kg^{-1}) and on day 2 for the reference pool (511.8 μmol kg^{-1}; fig 5).

DIC decreased over the course of the tide cycle on all 3 days for both pools and the Strait but the range observed in the tide pools was an order of magnitude greater and greatest on day 2 (1021.914 and 982.561 μmol kg^{-1} for the reference and treatment pools respectively versus 101.874 μmol kg^{-1} in the Strait). DIC decreased significantly less towards the end of the tidal cycle for day 3 in both pools and even less in the treatment pool with additional mussels than the reference pool (fig 6).

pCO2 was perhaps the most shocking of the carbonate chemistry values, particularly in light of ocean acidification. Each pool exhibited extreme ranges in pCO2 values on the order of 3 magnitudes with the greatest difference of 2031.046 and 1647.71 µatm on day1 for the reference and treatment pools, respectively. Yet the Strait did not experience as great a difference of 60.89 µatm on day 2 (fig 7).

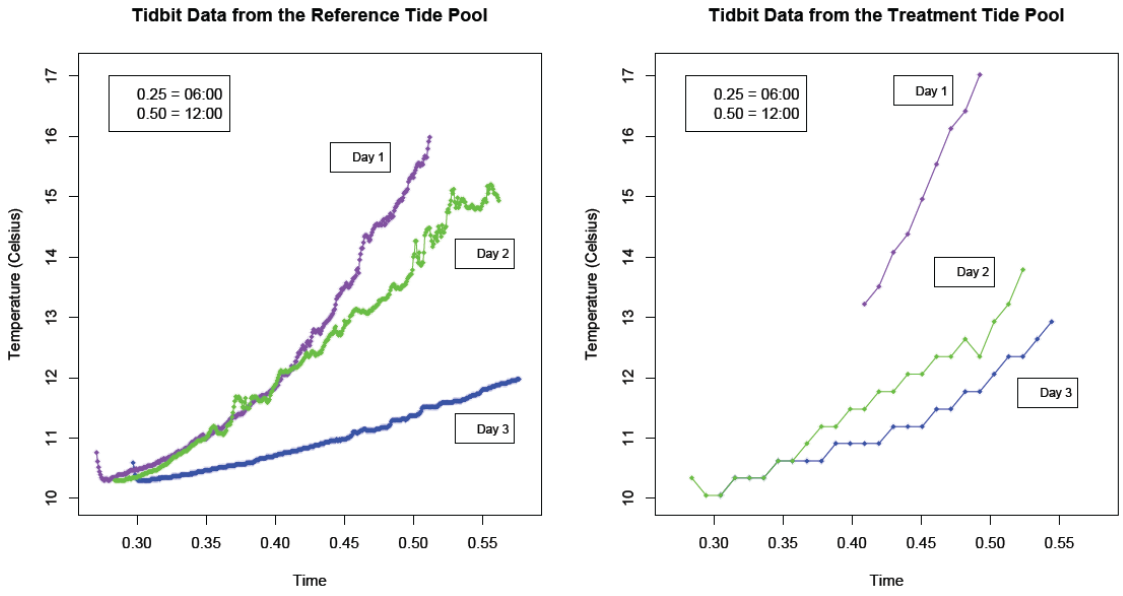


Figure 3. Temperature data from data logger (tidbit) from reference and treatment tide pools for all 3 days. Note for all R-based graphs, the time scale is defined in the legend (0.25 = 6:00am, 0.50 = 12:00pm).

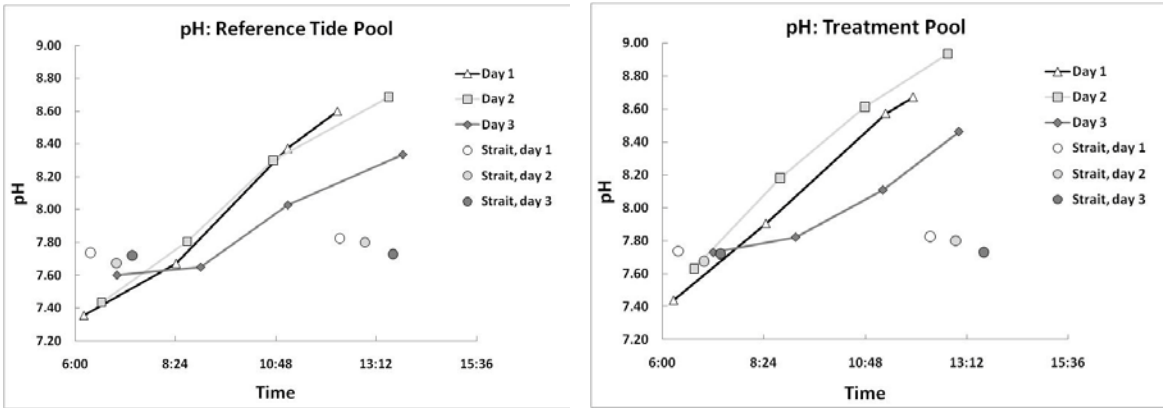


Figure 4. pH (total H^+ scale) over the course of the tidal cycle for the reference and treatment tide pools and the Haro Strait on all 3 days. Algal addition was on day 2, mussel addition was on day 3.

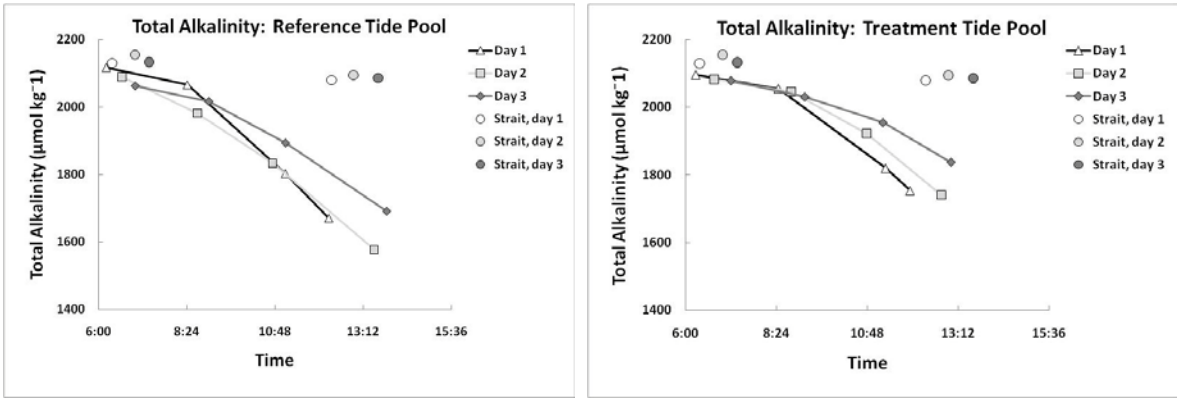


Figure 5. Total alkalinity of the reference and treatment tide pools and the Haro Strait over the course of a tidal cycle for all 3 days. Algal addition was on day 2, mussel addition was on day 3.

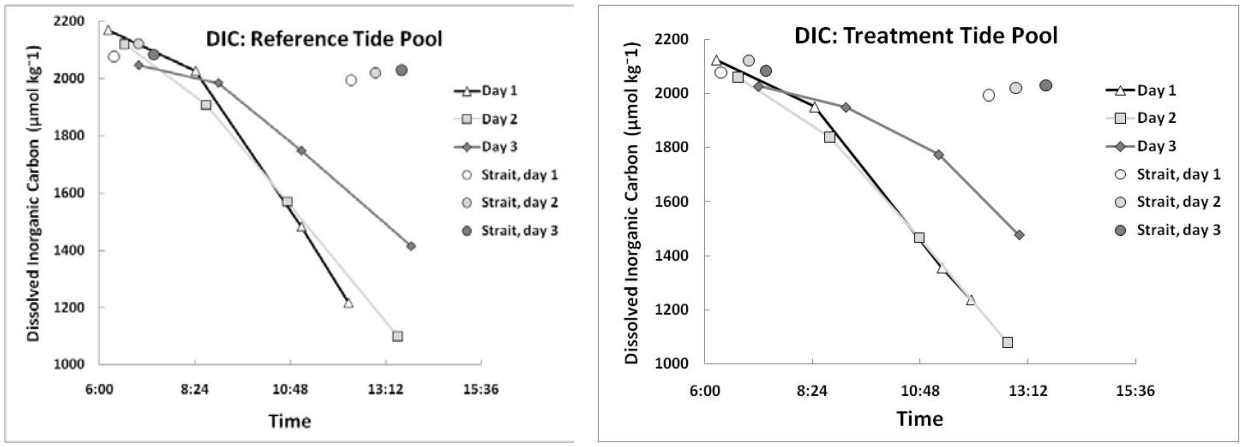


Figure 6. Total inorganic carbon (dissolved inorganic carbon, DIC) over the course of a tidal cycle for all three days of both tide pools and the Haro Strait. Algal addition was on day 2, mussel addition was on day 3.

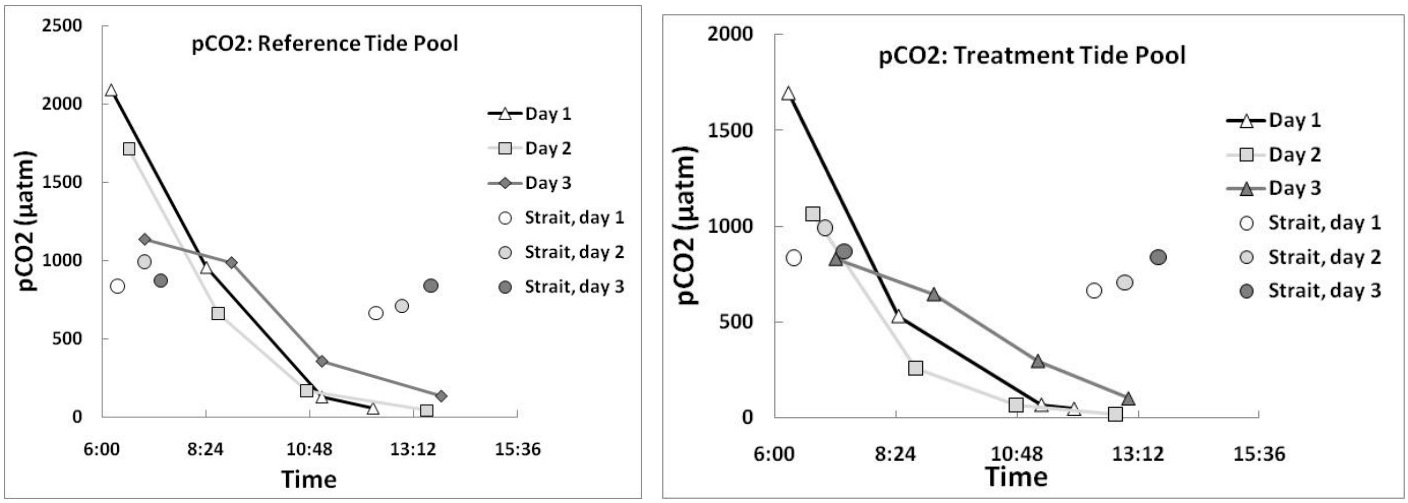
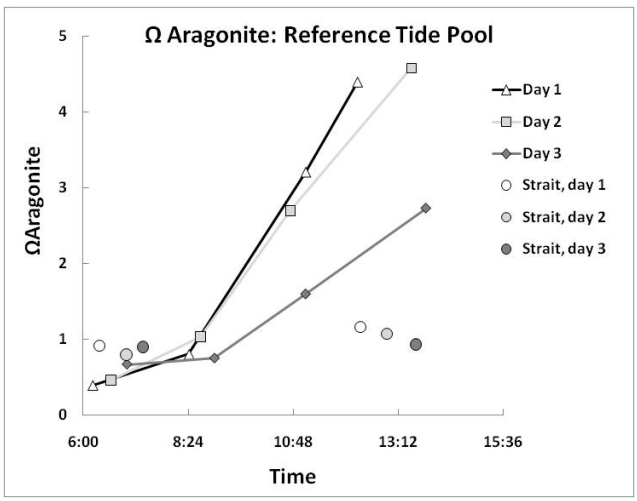


Figure 7. pCO2 over the course of a tidal cycle for three consecutive days for both tide pools and the Haro Strait. Algal addition was on day 2, mussel addition was on day 3.

Calcite and aragonite saturation states (ΩCa and ΩAr) followed the same trends as pH; as pH increased over the course of each tidal cycle each day, so too, did both saturation states (fig 8). Again, significant changes were observed in both tidal pools relative to the Strait. The largest difference in ΩCa was observed on day 2 for the Strait waters (0.428 units) versus 6.425 and 9.077 units for the reference and treatment pools respectively. Differences in ΩAr were not as great but still followed the same trend in range of maximum and minimum values between the Strait and the two tidal pools (0.273 versus 4.117 and 5.807 on day 2 for the Strait, reference and treatment waters respectively). At the earliest sampling times, both saturation states were below 1.0 in the



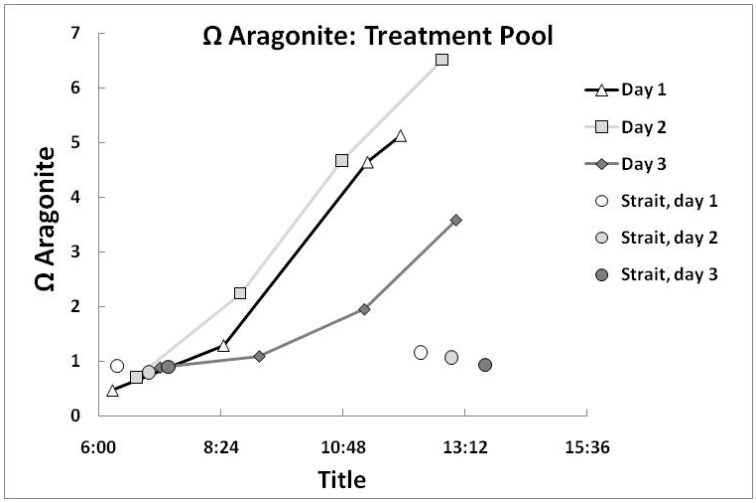


Figure 8. Aragonite saturation states for both tide pools and the Haro Strait over the course of a tidal cycle for 3 days. Algal addition was on day 2, mussel addition was on day 3. Because calcite saturation states followed the same trends (although at different values), data is not presented here – refer to table 2.

reference tide pool on days 1 and 2 and on day 3 only the ΩAr was below 1.0. In the treatment pool, both saturation states were below 1.0 on day 1, but on days 2 and 3 only the ΩAr was below 1.0. Conversely, the waters sampled from the Haro Strait were only undersaturated with respect to ΩAr, and undersaturation was observed on each day at the first time sampling. In addition, the saturation states of the Strait never got above 2.0, whereas the tide pools both experienced significantly higher saturation states.

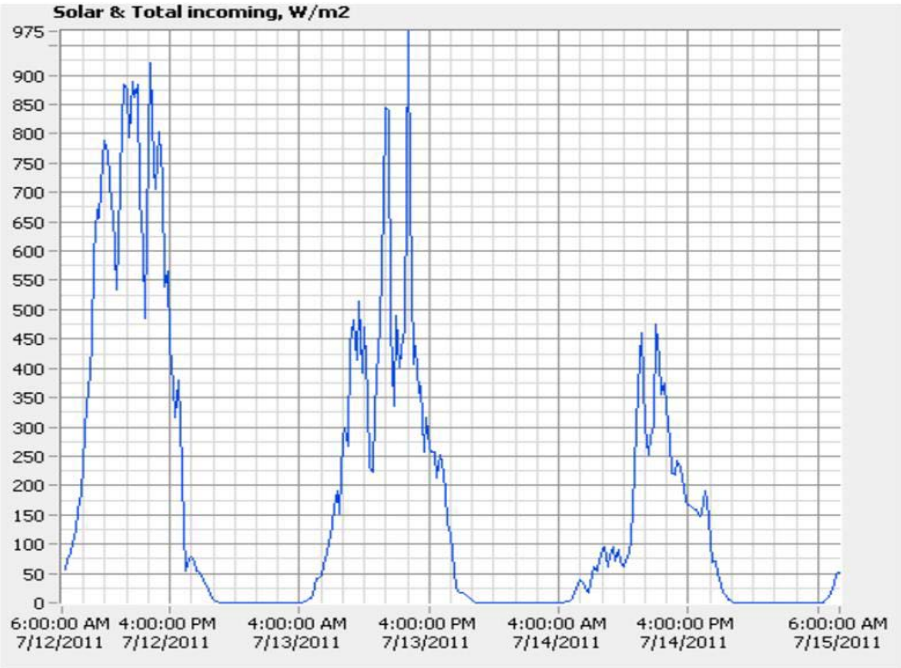


Figure 9. Solar radiation at Friday Harbor Labs on the days observations were made.

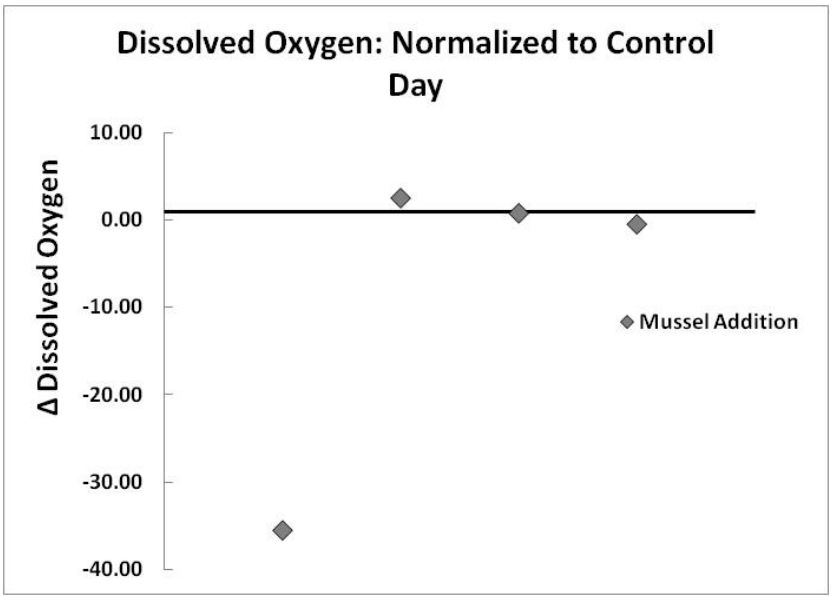


Figure 10. D.O. observed on the day of mussel addition normalized to control day (no manipulations made). Note that the x-axis represents a value of 1.0, or no change between the treatment and control days.

Upon the addition of mussels (day 3), D.O. did not change significantly over time despite the fact that the starting D.O. was significantly lower than on the control day (e.g., the non-manipulated day (fig 10). pH, however, did change between the two experimental days (fig 11). Upon addition of algae pH increased throughout the tidal cycle whereas upon the addition of mussels a decrease in pH was observed until the last time point.

Total alkalinity observed on the experimental days relative to the control day (day 1) fluctuated greatly and increased significantly on both experimental days from time points 2 to 3 (fig 12).

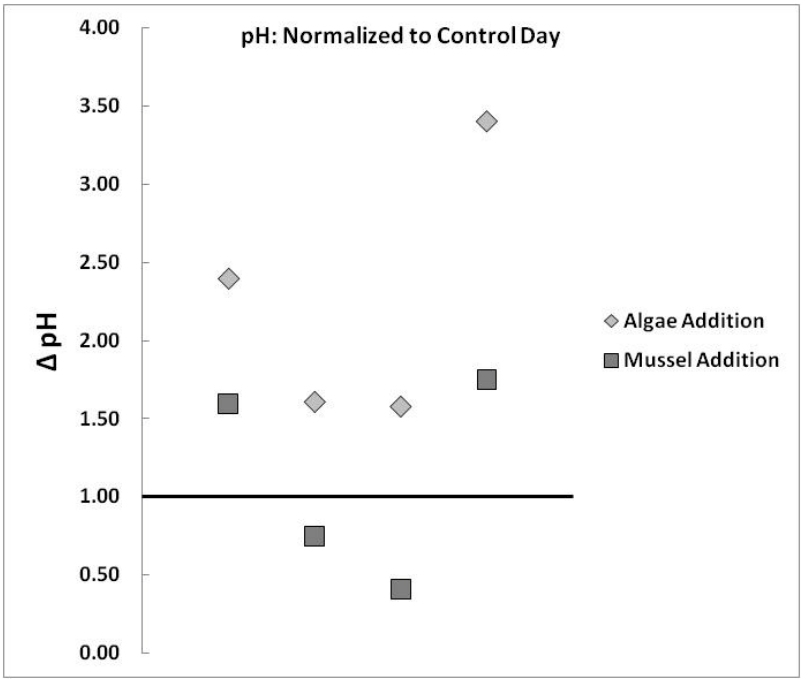


Figure 11. pH observed on the two experimental days normalized to the control day. Note that the x-axis represents a value of 1.0, or no change between the treatment and control days.

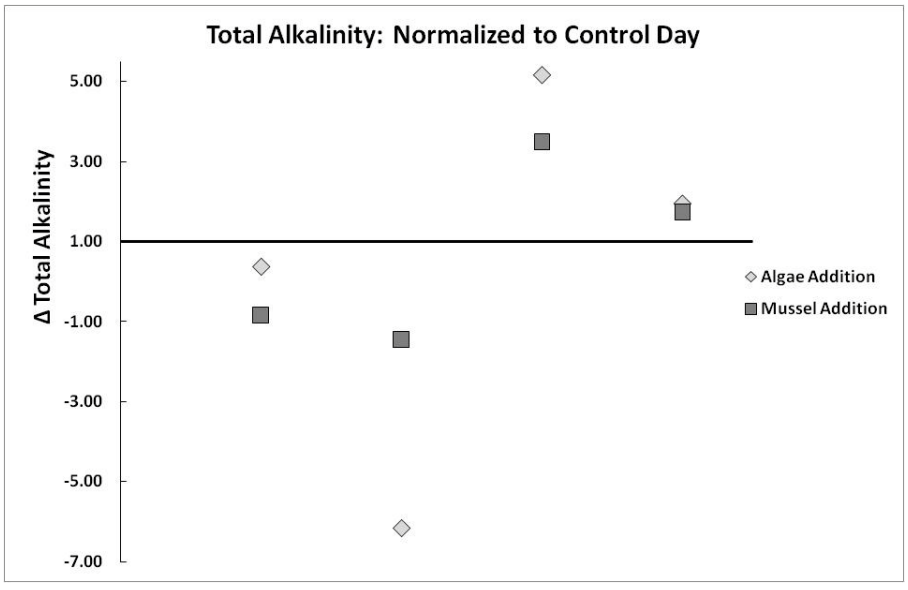


Figure 12. Total alkalinity observed on the two experimental days relative to the control day. Note that the x-axis represents a value of 1.0, or no change between the treatment and control days.

DIC on the experimental pools displayed a decrease on the algal addition day and an increase on the mussel addition day by the end of the tidal cycle (fig 13).

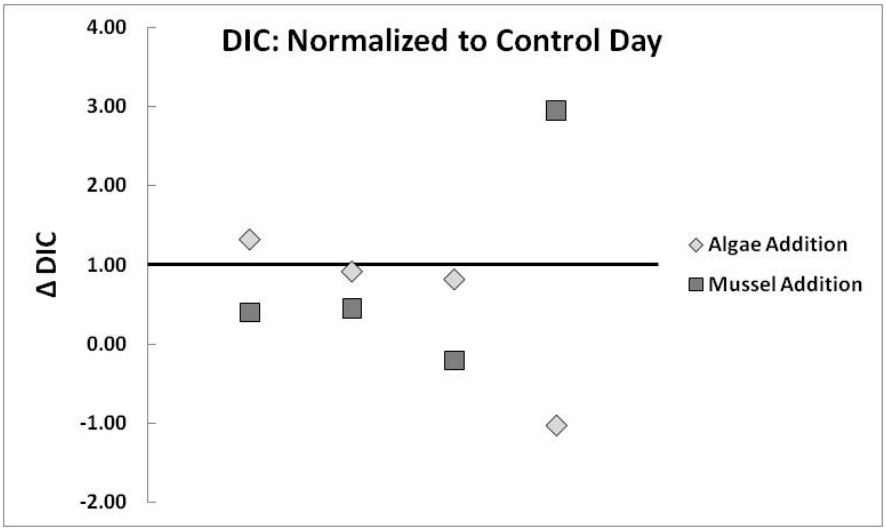


Figure 13. DIC for days 2 and 3 normalized to day 1 (control day). Note that the x-axis represents a value of 1.0, or no change between the treatment and control days.

pCO2, as mentioned above, but perhaps more clearly illustrated in the figure below, did not followed expected trends (fig 14). pCO2 mirrored DIC on the mussel addition day whereby there was an increase in pCO2 at the last sampling point. However, on the algal addition day, pCO2 also increased at time points 3 and 4, in contrast to DIC (fig 13).

Saturation states followed pH in terms of trends and therefore are not shown here.

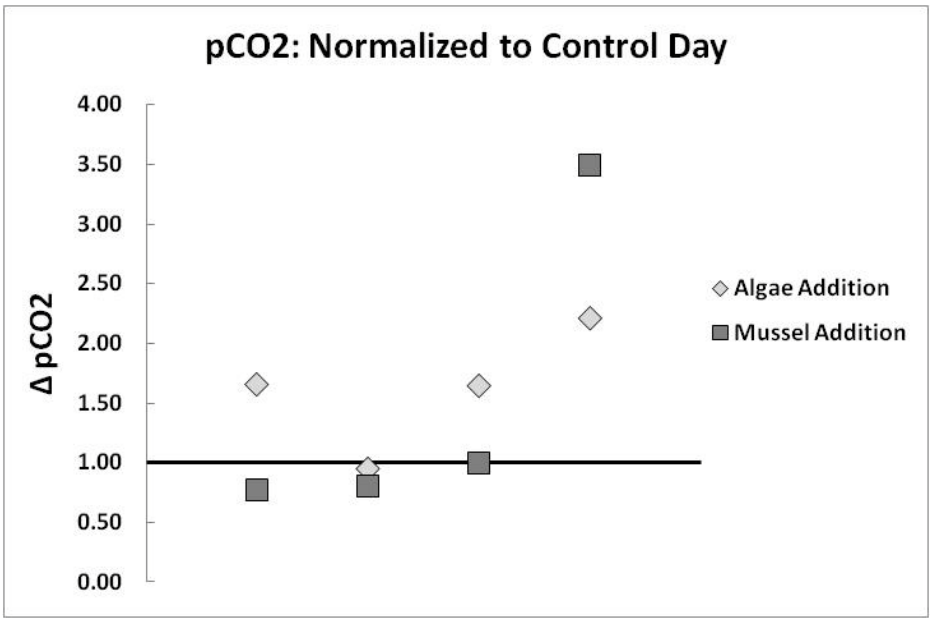


Figure 14. pCO2 of day 2 and 3 (algae and mussel addition, respectively) relative to day 1 (control day). Note that the x-axis represents a value of 1.0, or no change between the treatment and control days.

DISCUSSION

The greater temperatures, salinity, D.O., pH and lower pCO_2 and DIC in both pools on day 1 can all be explained by greater solar radiation that the tide pools experienced on day 1 relative to days 2 and 3. Photosynthetic rates increase with increasing solar radiation. *Ulva lactuca* has been shown to uptake oxygen at rates of up to 1.96 μmol O_2 m^{-2} s^{-1} (Longstaff et al 2002). Therefore, for the amount of *Ulva* sp. that we placed in the pool, maximum rates of O_2 uptake could have been approximately 2 μmol O_2 s^{-1}. The differences observed between tide pools on day 1(table 2) were most likely due to their different volume and biota.

The fact that D.O. did not decrease relative to day 1 (fig 2, 10) on the day mussels were added may be due to the fact that the mussels were not acclimated to the pool before the start of the experiment. The mussels were transported and immediately placed in the pool, thus they likely required some time before opening and respiration rates may have been suppressed. Perhaps with a longer tide exposure or a greater amount of biomass a respiration D.O. signal would have been present.

Trends in pH were expected whereby the addition of algae caused an increase in pH over the control day as the algae removed CO_2 from the tide pool. Macroalgae have been found in coastal areas where the pH has reached between 9 - 10 in rock tide pools (Middelboe and Hansen 2007, refs within). The addition of mussels may have caused an increase in CO_2 and therefore offset an increase in pH. Yet at the last sampling time point on the mussel addition day, the decrease in pH was less than unexpected relative to day 1 (fig 4, 11). Although speculative, this lack of pH decrease may be due to the fact that the day had lower solar radiation and colder temperatures, thus photosynthesis may have been 'slow' to overtake respiration earlier in the day. Further studies on changes in cloud cover and solar radiation to carbonate chemistry are needed.

Significant fluctuations in total alkalinity that were observed on the experimental days were unexpected, especially on the algal addition day (fig 5, 12). Addition or removal of CO_2 does not affect total alkalinity (Wolf-Gladrow et al 2007) and therefore some other processes must be taking place that significantly impact total alkalinity. Such processes may include the incorporation of phosphate by algae, which increases alkalinity, versus calcification, a process that decreases alkalinity (Wolf-Gladrow et al 2007). Various macroalgae (e.g., Fucus sp.) have been shown to have rapid rates of

phosphate uptake (Hurd and Dring 1991). More likely, the algae added to the tide pool shaded the coralline algae within the tide pool and thus the corallines reduced their calcification rates. The results of day 3 (mussel addition) were surprising in that a significant lack of an alkalinity decrease occurred by the third sampling point relative to day 1 when no additional calcifying organisms were added. It is possible that the tide pool did not have enough of a bacterial load to potentially uptake excretion products produced by the newly added mussels (e.g., ammonium). Further studies are required to validate this unexpected increase in total alkalinity (offsetting the overall decrease in alkalinity) and characterization of nutrient concentrations may help to further understand what processes may be contributing to total alkalinity.

DIC trends observed were expected (fig 6, 13). On the algal addition day the *Ulva* sp. were likely removing more DIC and thus the decrease in DIC relative to day 1 was expected (fig 13). On the mussel addition day DIC increased by the last sampling point, suggesting that mussel respiration was adding DIC to the tide pool by this time; DIC may not have increased earlier due to a short acclimation period whereby mussels had not opened up yet nor increased respiration rates (fig 13). Temperature also affects physiological rates; day 1 and 2 had higher temperatures relative to day 3 and thus respiration rates may have been lower earlier in the day on day 3 relative to days 1 and 2.

Unlike DIC, pCO2 did not follow the expected trends on the algal addition day (fig 7, 14). Algae remove pCO2, thus an increase in pCO2 at time points 3 and 4 relative to day 1 where no additional algae were added was unexpected. In addition, DIC on day 2 did not follow this trend; DIC decreased throughout the day relative to day 1 (fig 13). Although speculative, this discrepancy may be due to the fact the pCO2 was calculated

from CO2Calc whereas DIC was measured directly from water samples. CO2Calc was written for open ocean carbonate chemistry values (Andrew Dickson, *pers comm*) and therefore the calculated values may not be representative of the true measured values. pCO2 is also temperature dependent whereas DIC is not (Dickson et al 2007). In addition, changes in pCO2 may also be due to changes in photosynthetic or respiration rates over time. For example, algal photosynthesis is inhibited at high O2 concentrations (Turner and Brittain 1962). Thus the differences in temperature between days and between sampling time points may have been enough to cause these observed changes in pCO2.

Saturation states had similar trends to pH (fig 8 for saturation state trends, 11 for trends relative to control day) and thus were expected up until the last sampling time point on day 3 (mussel addition) where saturation states increased relative to day 1 (control). Because mussels respire and addition of CO2 decreases calcite and aragonite saturation states, it was expected that saturation states would decrease throughout the day, or increase less, relative to day 1. Other processes taking place within the pool or between the pools on each day may have complicated such a signal and perhaps adding additional respiring biomass might have resulted in a more significant signal.

There have been other studies on carbonate chemistry of tide pools (e.g., Daniel and Boyden 1975; Truchot and Duhamel-Jouve 1980; Morris and Taylor 1983 and Middelboe and Hansen 2007) yet some of these studies focused on changes in oxygen and/or the carbonate chemistry was often determined via pH measurements with a potentiometric probe or manual alkalinity titrations. This study incorporated methods that provide increased accuracy and precision in the carbonate parameters measured such

as spectrophotometric pH, automated total alkalinity and DIC determination by a gas non-dispersive infrared analyzer (Dickson et al 2007). This study found extreme values in carbonate chemistry over the course of a tidal cycle and values that varied significantly between pools and days. The main control on carbonate chemistry is certainly biological versus physical. It is important to point out that some of the fluctuations detected in pCO2 or O2 may also be due to changes in diffusion gradients with the atmosphere (given that concentrations of CO2 and O2 within the pools were so different from those of the atmosphere) and thus the presence or absence of wind that may affect diffusion rates could potentially confound expected trends. The physiological mechanisms underlying intertidal organisms' ability to cope with such dramatic fluctuations in both physical and chemical parameters have yet to be fully elucidated. The results of this study have important implications for ocean acidification experiments that use intertidal organisms both in terms of target carbonate chemistry values and fluctuations of those values. Many experiments use target values predicted for near-future conditions in the open ocean (e.g., pCO2 values of 1000 μatm) as opposed to the values found in this study (e.g., a pCO2 of 2000 μatm). In addition, many experiments have not incorporated the daily or diurnal fluctuations such organisms are experiencing (e.g., a pCO2 of 50 – 2000 μatm) and therefore may not be ecologically relevant. Future studies on tidal pool carbonate chemistry should include higher frequency of sampling, increased characterization of tide pool biota, characterization of nutrient concentrations and daily weather fluctuations as well as night time cycles in order to better understand the processes taking place within tide pools in the context of carbonate chemistry and to fully characterize the entire range of carbonate values experienced by the organisms.

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