Tidepools in Dead Man's Cove show large fluctuations in carbonate chemistry during the low tide in comparison to Haro Stait water

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ABSTRACT

During the low tide, tidepools are under different physio-chemical conditions than the coastal waters. This includes the carbonate chemistry, which is especially pertinent to study due to the threat of ocean acidification on carbonate chemistry. We examined the fluctuation in DIC, total alkalinity, pH, temperature, dissolved oxygen, and salinity in two tide pools in Dead Man's Cove, San Juan Island, WA over the course of the low tide. To better understand the relative contributions of photosynthesizers and respirers to changes in carbonate chemistry, we added *Ulva* to one pool on the second day, and *Mytilus trossulus* on the third day. During the low tide, tidepools saw a decrease in DIC and alkalinity, and an increase in pH, temperature, and dissolved oxygen. There is evidence of a small signal of decrease in DIC due to *Ulva* addition and increased DIC due to *Mytilus* addition. Tidepools experience huge swings in carbonate chemistry that suggests an ability of the organisms that inhabit the pools to cope with large changes in pH over the course of the day.

INTRODUCTION

As ocean acidification changes the carbonate chemistry of the ocean, to understand what conditions organisms will face under future warming scenarios, it is important to understand the chemistry of the current system. The San Juan Islands are a dynamic area of different water masses mixing, all of which influences the chemistry. While the open ocean has been subjected to many studies of carbonate chemistry

monitoring (Feely *et al*., 2010), there is less information on what the chemical composition is on the coast.

One of the areas predicted to be more variable on the coast are tidepools formed in the intertidal. Tidepools, formed as isolated pools when the tide goes out, have already been shown to undergo large temperature, salinity, and even nutrient shifts that organisms out of the pool do not face. Due to the closed system and abundance of respirers and photosynthesizers, it is also possible that these pools undergo fluctuations in carbon chemistry during a tidal cycle. Dissolved inorganic carbon (DIC) is comprised of CO_2, HCO_3^- , and CO_3^{2-}. Photosynthetic algae primary use HCO_3^- as their carbon source, thus drawing down the DIC in a pool (Murru and Sandgren 2004). Respirers release CO2, increasing DIC in a pool. Total alkalinity defines all of the alkaline elements in a pool, including carbonate ions. As organisms calcify, they precipitate calcium carbonate out of the pool decreasing alkalinity. Nitrogen, in the form of ammonia, produced from animal excretion works to raise alkalinity (Wolf-Gladrow *et al.*, 2007). A decrease in alkalinity and DIC increases the pH of the water.

These fluctuations in carbon chemistry have been recorded in tide pools. Daniel and Boyden (1975) report an increase in pH and decrease in CO_2 in a tide pool in the UK over the course of the day when the pool is exposed. He attributes the presence of photosynthesizers to these changes. Truchot and Duhamel-Jouve (1980) observed the same pattern in tidepools on the English Channel. Additional, they measured total alkalinity and pCO_2 and found that in tide pools during the day total alkalinity and pCO_2 decreased. However during the night, they recorded the opposite pattern, further showing the relative influences of photosynthesis and respiration. Morris and Taylor (1983) found

pH reaches over 9 in the summer in tidepools on the west coast of Scotland. These studies show a similar pattern in the direction of chemistry changes.

We sought to observe how carbon chemistry in a tidepool changes over the low tide in tidepools in Dead Man's Cove, San Juan Island, WA. These values are informative of the range of values tidepool organisms in this area experience each day. We chose two tide pools located at similar tide heights and measured temperature, dissolved O_2, salinity, pH, alkalinity, and DIC over a tidal cycle in each pool, and the strait water that fills the pools on the high tide. The tide pools will show greater increases in temperature, salinity, pH, and dissolved oxygen in comparison to the strait due to lack of tidal flushing while the presence of organisms will result in large decreases in TA and DIC. Differences between the pools may be due to either differences in size, or differences in species composition. To better understand the relative contributions of photosynthesizers and respirers and further understand how tidepools fluctuate in a day, we changed one tide pool by adding *Ulva* on day 2 and *Mytilus* on day 3. The *Ulva* addition will decrease the DIC and alkalinity as photosynthesis would consumer carbon in the water. The *Mytilus* addition will lead to decreases in total alkalinity as the organisms calcify and increase DIC as they respire. Overall, the effect of adding biomass will increase the daily fluctuations.

METHODS

Study Site

We conducted the study at Dead Man's Cove, San Juan Island, WA (N 48˚30.837, W123˚08.763). Dead Man's Cove is on the Haro Strait that runs between Vancouver Island and San Juan Island (Figure 1). We chose this site because it had a variety of large

pools where water removal for samples would not affect pool dynamics. We chose the two tidepools to be as similar as possible in tidal height in order to ensure the tidal cycle affected the pool at the same time of day. The pools were located in the *Fucus* zone so they would be exposed for a good portion of the low tide. Within this tidal height, we chose pools with similar biota. However, due to the lack of abundance of tidepools, one pool was larger than the other. The study ran from 12-July-2011 to 14-July-2011.

We measured volume of the pools by taking multiple measurements of depth and length with a meter stick, and averaging these values to calculate a coarse estimate of volume. We used stadia rods and a hand level to determine the tidal height of each tidepool. To estimate the biota of the pools we visually assessed percent cover of macroalgae and estimating mobiles on the scale of present (1), few (<10), many (10-50), and abundant (>50).

Quantifying Chemical composition over a tidal cycle

To quantify carbonate chemistry changes in a tidepool over the low tide to understand the extremes tidepool organisms are exposed to, we collected water samples during four intervals spaced 120 minutes apart during the time the tidepool was exposed. Additionally, we took samples from the shore, greater than 500 m from the tide pools, in the strait during the first and last sampling periods, as the strait water shows the conditions the tidepool organisms are exposed to before the tide went out and after the tide came in.

To collect water samples, we used a rubber stopper with an air hole and tygon tubing running from the top of the stopper to the bottom of the 500 mL Schott Duran

borosilicate glass bottle to prevent large macro-algal pieces from getting into the sample bottle (Daniel and Boyden 1975). We collected all water samples as outlined in SOP1 (Dickson *et al.* 2007). A glass stopper applied with Apiezon® L grease formed the airtight seal following the addition of 100µL saturated $HgCl_2$. We stored the bottles in a dark cooler prior to analysis.

Every hour the tidepools were exposed during the low tide, we measured temperature, salinity, and dissolved oxygen, except on day 2 when the meter was no dissolved oxygen meter was available, only temperature was measured (Hack Instruments). Additionally, we placed temperature tidbits (Onset) in the tidepools to record temperature while the tidepools were exposed.

We then analyzed the samples in the lab for total alkalinity, dissolved inorganic carbon, and pH (Dickson *et al.* 2007). We used an Agilent automatic titrator (model 34970) to measure total alkalinity and measured DIC with a Licor infra –red analyzer. We checked values from these machines for precision and accuracy by running Certified Reference Materials (CRM, Batch 111, A_T = 2222.8 µmol kg^{-1} DIC = 2045.7 µmol kg^{-1}, A. Dickson, Scripps Institute of Oceanography).

pH

We measured pH using an Ocean Optics spectrophotometer using optical cells with a 10 cm path-length following the procedure of SOP6b (Dickson *et al*., 2007). Preliminary measurements of tidepool pH taken earlier in the class alerted us to the potential of high pH's in tidepools. Therefore, we utilized two different dyes to measure

pH. *m*-cresol purple can provide an accurate pH over the range of 7.4 to 8.2 and thymol blue provides pH measurements for pH's of 8 to 9.

We mixed a 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 using the constants for Lueker *et al*. (2000), Dickson (1990b), and Wanninkhof (1992). These calculated values best matched the *m*-cresol purple values (Figure 2). 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 scale (mol $[H^+]$/kgSW).

Tidepool Manipulations

To better understand the extremes that tidepool organisms are faced with and tease apart the relative contributions of photosynthesizing and respiring organisms, we added organisms to one tidepool on day 2 and day 3. We chose to add organisms to the smaller tidepool of the two in order to detect a signal. This tidepool will be referred to as the 'treatment' pool and the unmanipulated pool will be referred to as the 'reference' pool. On day 2, 1.014 m^2 of *Ulva* was added to the pool to measure tidepool chemistry when dominated by a highly photosynthetic organism (see appendix 1 for area calculation). We removed the *Ulva* at the end of the day. On day 3, we added 278.1 g of *Mytilus trossulus* to the pool to ascertain if an extra respirery load would affect the pool's

chemistry. We collected *M. trossulus* from the dock at Argyle Creek (48˚ 31'09.92"N, 123˚ 00'47.68"W) six days prior to the start of the experiment. We then housed the mussels in a flow through aquaria at Friday Harbor Labs and removed all encrusting biota with a file. We determined buoyant weight 24 hours prior to mussel addition by suspending mussels in seawater from a bottom-loading balance (Davies 1989).

To compare pools between days, we compared the difference between the manipulated and un-manipulated pool on the *Ulva* and *Mytilus* addition days to the difference between the pools on the control day when we made no biomass additions, expressed in the following equation:

$$\frac{treatment_{day2/day3} - reference_{day2/day3}}{treatment_{day1} - reference_{day1}} \quad (1)$$

This calculation makes the assumption the difference between the two pools is constant, and therefore any deviation between the pools is due to the biomass changes we made.

RESULTS

Tidepool Characterization

The un-manipulated pool had a volume of 0.41 m^3 while the manipulated pool's volume was 0.10 m^3. The pools had some mobile species overlap. For example both pools had *Littorines, Nucella*, limpets, and hermit crabs, but these species were present in different abundances (Table 1). Similarly, coralline algae dominated both pools, but otherwise the pools had different algae profiles (Table 2).

The three sampling days occurred on the three days leading up to the spring tide (July 14), and there was only a small variation in the tide cycle with respect to tide height over the days sampled (Figure 3A). Light levels measured at the Friday Harbor Labs

Weather Station, 10.9 km northeast of the study site had similar magnitudes of irradiance on day 1 and day 2, but had a reduced irradiance on day 3 (Figure 3B).

For both pools, the temperature was greatest on day 1, and lowest on day 3. The treatment pool experienced greater temperatures on day 1 and day 3 than the reference pool, but lower temperatures on day 2 than the reference pool (Figure 4).

Dissolved oxygen (DO) increased in both pools and the strait with time. However, the meter only measured DO to 200% saturation. The tidepools reached 200% halfway into the low tide, and therefore, we do not know whether DO continued to increase with time or leveled out above 200%. Salinity remained fairly constant in the two pools. The reference pool experienced salinities ranging from 30 to 31.3 while the treatment pool's salinities ranged from 30.1 to 31.5.

Carbonate Chemistry

Over all three days, the Haro strait waters had a greater DIC (Total Carbon) than either of the treatment pools. DIC decreased in both pools and the strait, but there was a much greater decrease in the pools than in the strait. The pools showed a similar degree of decrease and pattern of decrease with time (Figure 5).

Total alkalinity decreased with time across all treatments and days. The Haro Strait had a greater total alkalinity than both the reference tidepool and treatment tidepool. The reference tidepool, the larger of the two tidepools showed a greater decrease in total alkalinity than the treatment tidepool across all days. The treatment tidepool showed the greatest decrease in total alkalinity on the control day, and the least decrease on the *Mytilus* addition day (Figure 6).

pH increased with the time the tidepools were separated from the strait. There was a slight increase in the strait waters during this time period on day 1 and day 2, but not on day 3. The tidepools both showed the greatest increase in pH on day 1 (control day) and day 2 (*Ulva* addition). For both pools on day 3 (*Mytilus* addition day) the pH increase between the first two time points was very small in comparison to the rate of increase between the next two time points (Figure 7).

Biomass Additions

We calculated the ratio of the difference between the two pools on the addition day to the difference between the two pools on the control day to see if we could detect a signal from the biomass addition. If there was no difference in the ratio between pools from the control day to the addition day, the ratio is equal to 1. If the treatment pool has a larger value in comparison to the reference pool, then the change is positive. If the treatment pool has a smaller value in comparison to the reference pool, then there is a negative change.

On day 2, the *Ulva* addition day, DIC did not differ much from the control day, until the last time point when DIC in the treatment pool increased with respect to the reference pool. On day 3, the *Mytilus* addition day, there was a similar pattern, however DIC was slightly lower in the treatment pool and then increased with respect to the reference pool (Figure 8A).

Total alkalinity showed great variability within a day, however, this pattern of variability was conserved over both addition days. At the first two time points, the

addition pools had a smaller total alkalinity than the control pool, but at the later two times points, they had a greater total alkalinity (Figure 8B).

pH was consistently greater in the manipulated pool on the *Ulva* addition day with the greatest difference at the last time point. On the *Mytilus* addition day, the treatment pool had a greater pH at the first time point, a smaller pH at the next two time points, and a greater pH at the final time point (Figure 8C).

Aragonite saturation state followed a similar pattern as pH. The treatment pool had a consistently greater Ω_{arag} than the control pool at all time points on the *Ulva* addition day. However on the *Mytilus* addition day, the Ω_{arag} change fluctuated around 1 showing no distinct pattern (Figure 9D). Calcite saturation state (Ω_{ca}) followed the same pattern as Ω_{ara}.

DISCUSSION

While our data can only be applied to describe the two tidepools we measured, they still give a glimpse of what occurs in a tidepool over the low tide. A tidepool experiences drastic differences in DIC, and total alkalinity, and increases in pH and Ω_{arag} in comparison to the nearby intertidal water during a daytime low tide in the summer. The tidepools experience these extreme deviations from the nearby water even when they are different sizes with different species compositions.

These large deviations in DIC, alkalinity, and pH from the strait are consistent with studies of tidepool chemistry in other areas of the world (Truchot and Duhamel Jouve 1980, Morris and Taylor 1983, Daniels and Boyden 1975) that also found a drop in DIC and total alkalinity and rise in pH during the day. Calcifyers and photosynthesizers in the tidepools decrease alkalinity because they drawn carbonate, and bicarbonate out of

the system respectively (Wolf-Gladrow *et al*., 2007). The high abundance of calcifying corallines, limpets, snails and barnacles in both tidepools may draw down the carbonate concentration. Algal photosynthesis decreases bicarbonate and carbon dioxide. During the day when the sun is out, rate of photosynthesis increases causing the alkalinity to decrease more. Since the tidepool is isolated without access to new water, alkalinity becomes much lower in the pool than in the strait.

DIC was slightly less in the smaller tidepool than the larger tidepool until the end of the day. Both pools followed the same downward sloping trend with time of exposure. A reduction in DIC indicates a decline in one of the inorganic carbon species that comprise DIC. Photosynthesizers will lead to a decline in DIC because algae will take up carbon dioxide, bicarbonate, or both (Murru and Sandgren, 2004; Hurd *et al.,* 2009). The rate of DIC decline increases with time. This may be due to saturating light levels for photosynthesis not being reached until later in the day.

A decrease in alkalinity and DIC will increase pH. Even though warmer temperatures lower pH, both pools showed an increase in pH over the low tide, which points to the changes in carbon chemistry detected in alkalinity and DIC overwhelming any temperature effects on pH. pH was lower in the larger reference tidepool than the treatment tidepool on all days. As seawater can buffer pH (Archer *et al*., 1997) it is possible that the reference pool, with its larger volume, was better able to buffer pH changes.

Over all measured parameters (DIC, alkalinity, pH), values on day 3 were consistently lower in both pools. This may be due to a weather effect, as light levels and temperature were also lower. Lower light and lower temperatures would result in less

photosynthesis and less respiration, and thus draw down alkalinity and DIC less, changing pH less. Year round studies on tidepools show the change in alkalinity and DIC over a tide cycle during the winter is less severe than in the summer, due to lower light and temperature in the winter months (Middelboe and Hansen 2007). Thus on a smaller scale of within a month, colder, darker days will decrease the magnitude of changes in the pool.

The magnitude of the differences in carbonate chemistry in tidepools suggests tidepool organisms can cope with large changes in pH. pH changed by more than 1 pH unit over just six hours in the tidepool, while only a slight change occurred in the strait. This pH change spans the range of aragonite under-saturation, where calcification cannot occur, to super saturation of aragonite where calcification is possible. These results suggest both the enhanced ability of these organisms to deal with environmental changes in pH while keeping their internal pH constant as well as the ability to remain calcified under a large range of pH's. Carbonate chemistry may help structure the biota found in tidepools. For example, *Enteromorpha intestinalis* is able to deal with extreme changes in pH, which may explain part of why it can inhabit high tidepools (Gross-Custard *et al.,* 1979).

The normalization results show slight effects of the biomass additions that are most strongly seen in the changes in DIC. For the last time point of DIC, the addition of *Ulva* decreases DIC and the addition of mussels increases DIC as predicted. It is possible it took six hours for this signal to be seen due to organism acclimation time. The mussels were transported to the site in a bucket and might have needed that much time to open up

and begin respiring to increase DIC. The *Ulva* may have been stressed from being pulled up and thrown in a tidepool after being emerged on the rocks.

While the raw alkalinity data shows a clear difference between days in the treatment pool, this clear pattern is not seen in the normalization calculation. Rather, both days show a large jump from alkalinity being decreased in the treatment pool to increased with respect to the reference pool. I would predict the addition of a photosynthesizer on day 2 would decrease alkalinity relative to the control day while the addition of a calcifier on day 3 would also reduce alkalinity. However, these reductions are only seen in the first two time points where we believe the organisms may not be fully acclimated and thus not fully functioning. Therefore it is unclear of how biomass additions affect alkalinity. Thus the variability of alkalinity from day to day may be due to physical factors such as temperature or sunlight affecting physiological processes. In the treatment tidepool, on all days, the decrease in alkalinity from the first to the second sampling timepoint is the same. During this time I believe the photosynthesis and respiration are lower than the rest of the day due to lower light and lower temperature. After the initial alkalinity change, the lowest drop in alkalinity occurs on day 1, the day with the greatest temperature. The smallest decline in alkalinity is on day 3, the coldest day. While total alkalinity is temperature independent, it is possible that there are temperature effects on the organism's rates of photosynthesis, respiration, and calcification which affect alkalinity. Temperature on day 1 and day 3 was greater in the treatment pool, but on day 2 temperature was greater in the reference pool. Thus in normalizing the data to day 1 differences between pools, the assumption that these

differences will be the same through time is false. It is possible the different biotas in the pools are affected differently by temperature obscuring the alkalinity signal.

pH and aragonite saturation was elevated in the *Ulva* addition treatment in comparison to the control day. This matches what I predicted, as *Ulva*, removed carbon through photosynthesis increasing pH, which increases Ω_{arag}. Consistent with the DIC deviations, pH is most different from the control day at the last time point. However, pH does not follow the patterns predicted for the *Mytilus* addition. Mussel respiration should release CO_2 reducing the pH. Mussels calcify reducing pH. While the middle time points show a decreased pH in the *Mytilus* addition with respect to the control, the last time point, where I concluded from DIC is where the mussels opened up the most after taking time to become acclimated, differs from the expected pattern. pH is temperature depended, therefore, as noted earlier, the lack of consistency between temperature and tidepool between days may hide the biomass addition effect.

Overall, either not enough biomass was added to detect a clear signal, or changing the biological load in a tidepool does not significantly affect its chemistry. To further tease apart this relationship, future experiments could add more biomass to the pools. Another potential source of error is that the biomass additions were not in the pools long enough to detect a clear signal. Moving an organism causes it stress, and they may not perform at their optimum until they are better acclimated to their system.

Furthermore normalization graphs also point to variability of the system when considering the first time point on each graph. The first water samples were taken immediately following the biomass additions, thus these points should not differ from each other between days and therefore should equal 1. The difference between the values

of DIC, alkalinity, and pH at the initial time points shows that in the absence of manipulation, the differences between the two tidepools are not constant from one day to the next.

Truchot and Duhamel-Jouve (1980) saw the same patterns in carbonate chemistry during the day, and the opposite patterns at night. The Pacific Northwest experiences low tides that expose tidepools during the day in the summer and at night in the winter. It is possible that during the winter, these tidepools experience very low pH. Future experimentation could examine how carbonate chemistry differs between the summer and winter nighttime low tides. A greater time series of the carbonate chemistry fluctuations in tide pools provides a fuller picture of what conditions tidepool organisms are exposed to. This has implications for their calcification mechanisms, and internal pH regulation. This time series would also be informative in designing experiments to understand how future climate predictions will affect tidepool organisms.

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FIGURE AND TABLES

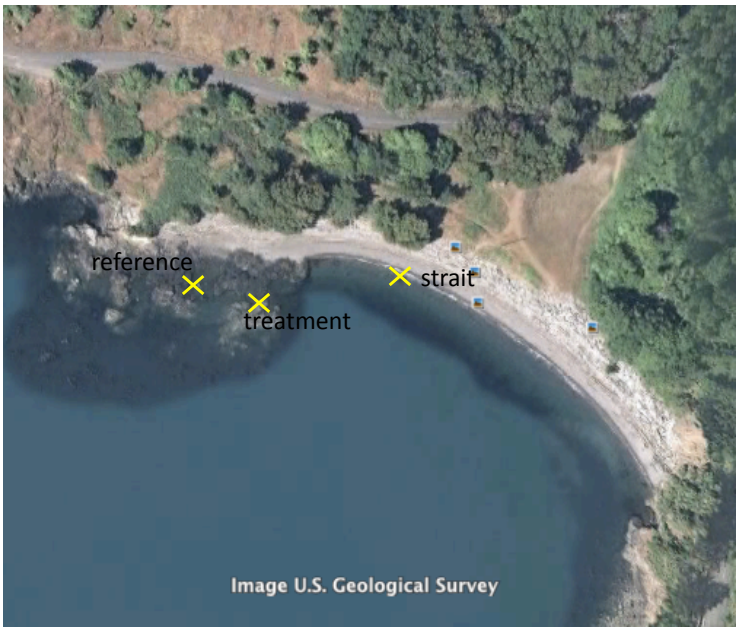


Figure 1: Location of sampling sites in Dead Man's Cove, San Juan Island, WA (N 48˚30.837, W123˚08.763). An X marks the approximate location of the two tidepools, and the location in the strait we collected a sample.

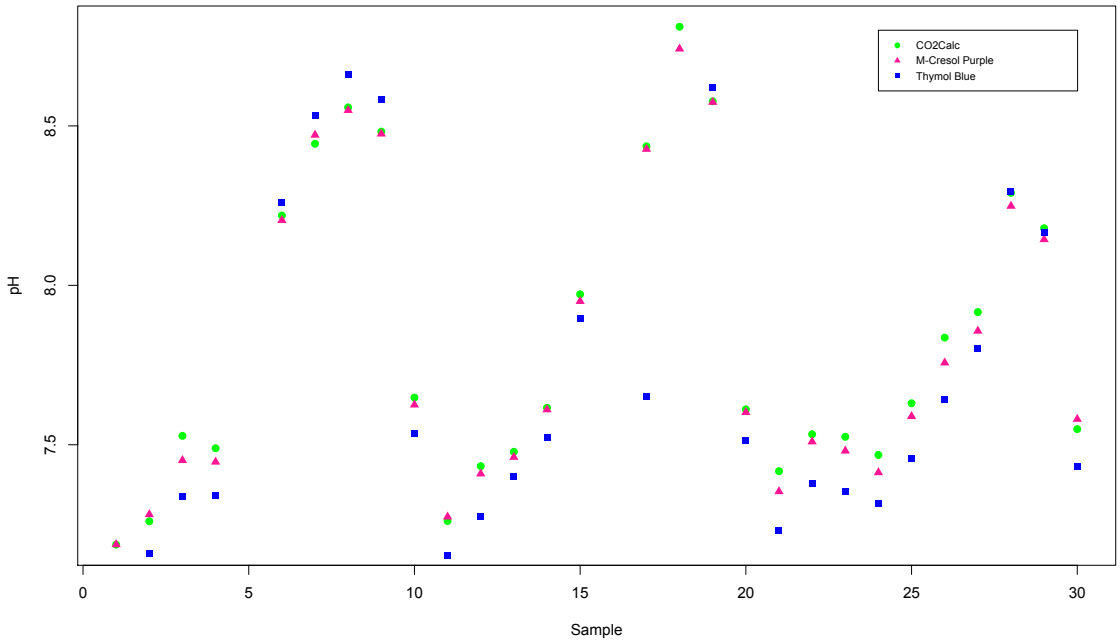


Figure 2: pH values of each sample measured at 25˚C from thymol blue dye, *m*-cresol purple dye, and CO2Calc. All values tracked each other with the *m*-cresol purple and CO2calc values being closest.

Table 1: Mobile abundance in the two tidepools (Few = <10, Many = 10-50, Abundant = >50).

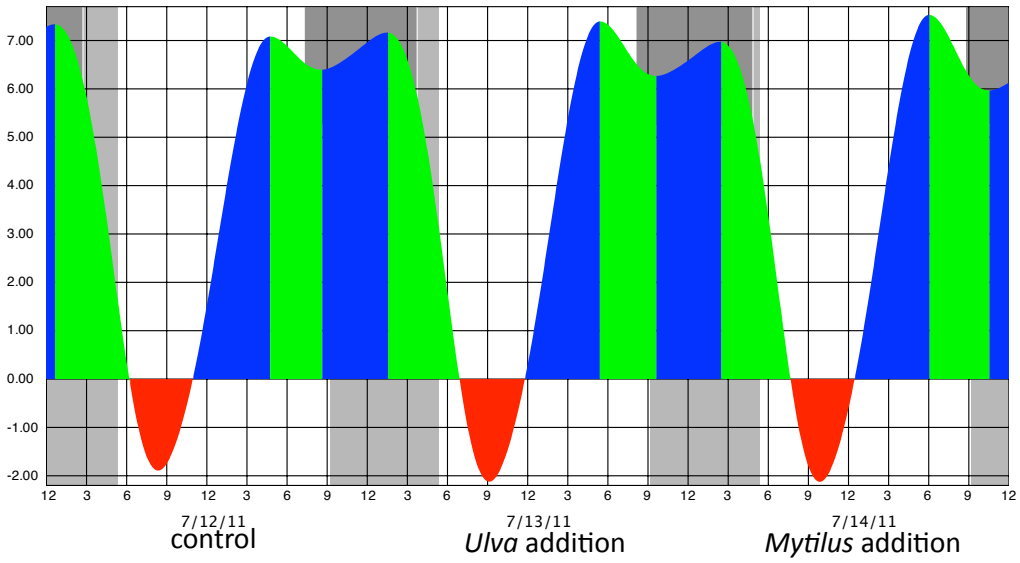
Tidepool	Present	Few	Many	Abundant
Manipulated		chiton spp., *Semibalanus cariosus*, amphipod spp., *Calliostoma spp*., *Littorina sitkana*, *Nucella spp*.		Medium and large *Pagarus spp*., *Lottia spp*. (at least 2 different kinds)
Un-manipulated	nemertean worm, *Katharina tunicata*	*Hemigrapsus nudus*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

complex

Table 2: Algal composition of the two tidepools.

Tidepool	Species	Percent Cover
Manipulated	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 foccosa	<1%
Un-manipulated	Crustose coralline	10%
	Corallina vancouveriensis and *Bossiella* spp.	55%
	Fucus distichus (loose)	5%
	Odonthalia foccosa	30%
	Prionitis lanceolata	<5%
	Leathesia marina	<5%
	Soranthera ulvoidea	<1%

A



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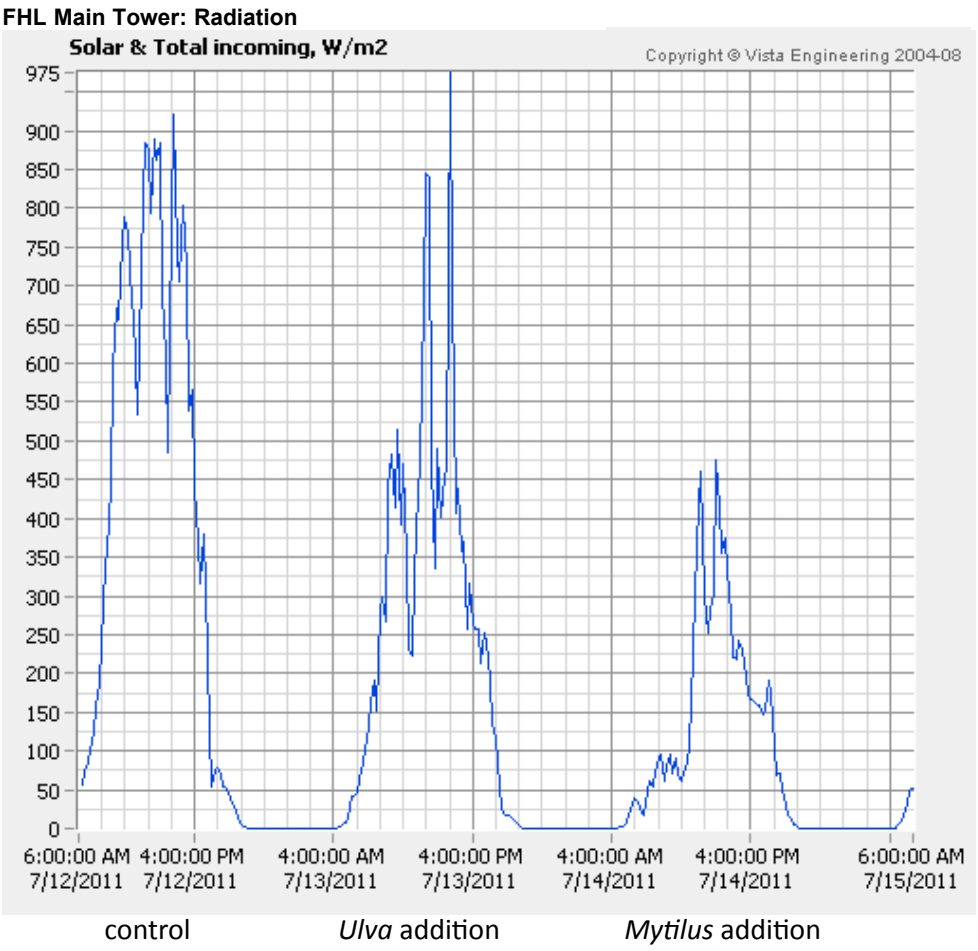

Figure 3: (A) Tidal height data for each sample day taken from Kanaka Bay, San Juan Island, WA from Mr. Tides Version 3. (B) Underwater light levels for each sample day taken at the FHL Labs weather station.

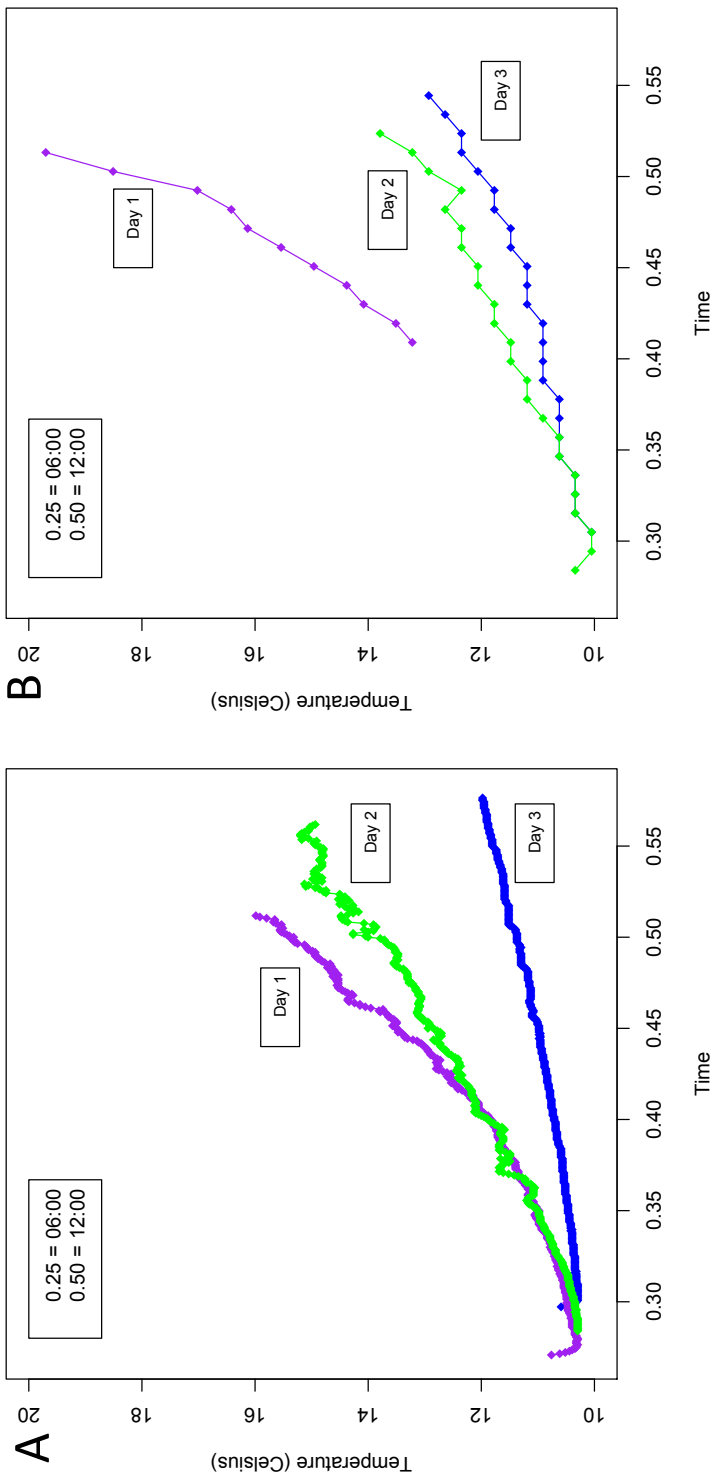

Figure 4: Temperature data from the reference pool measured every minute (A) and the treatment pool measured every 15 minutes (B) on each sampling day expressed as a fraction of the time of day. Temperature was greatest throughout the day on day 1, while day 3 had substantially lower temperatures than the other two days.

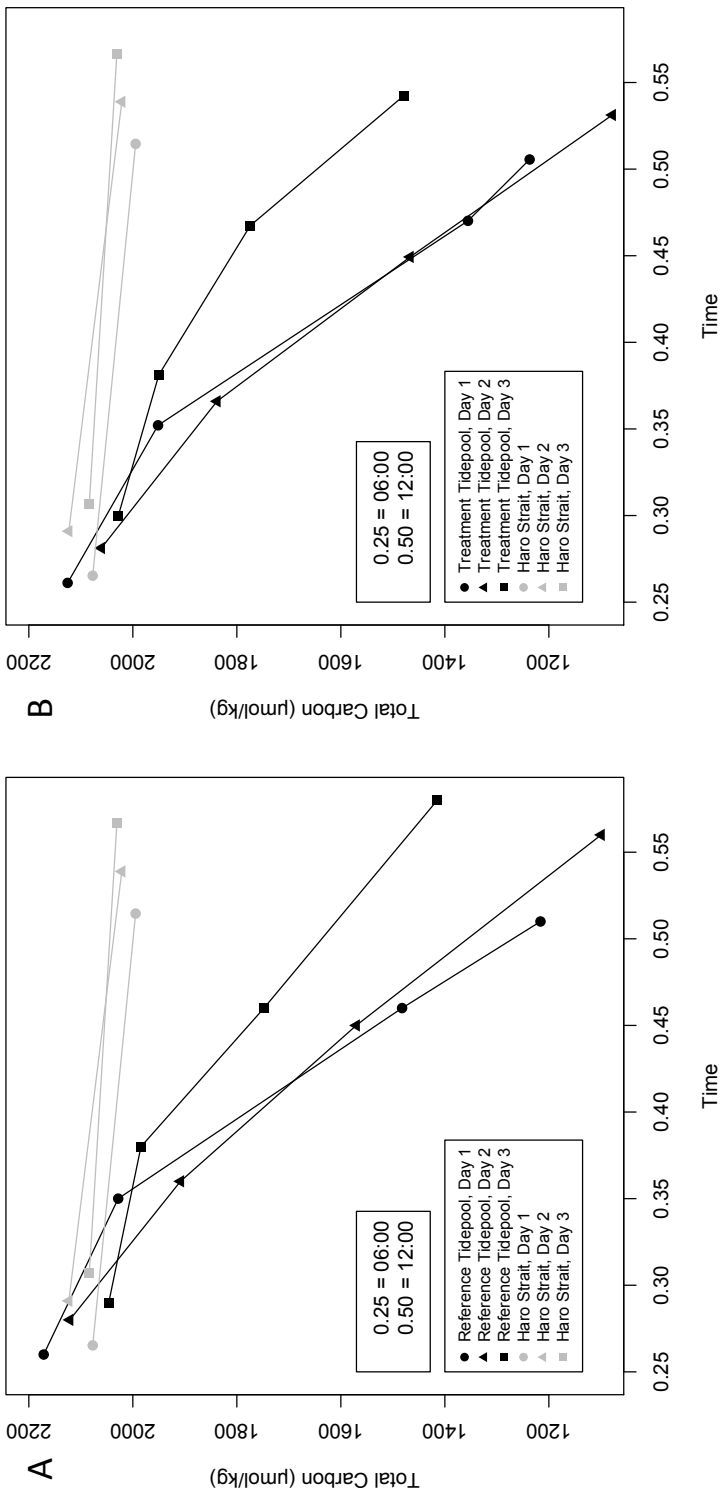

Figure 5: Total carbon in the reference pool (A) and the treatment pool (B) for the control day (Day 1), *Ulva* addition day (Day 2) and *Mytilus* addition day (Day 3) at four time points measured over the low tide. Time is expressed as a fraction of the day, where 0.25 corresponds to 6 am and 0.5 to 12 pm.

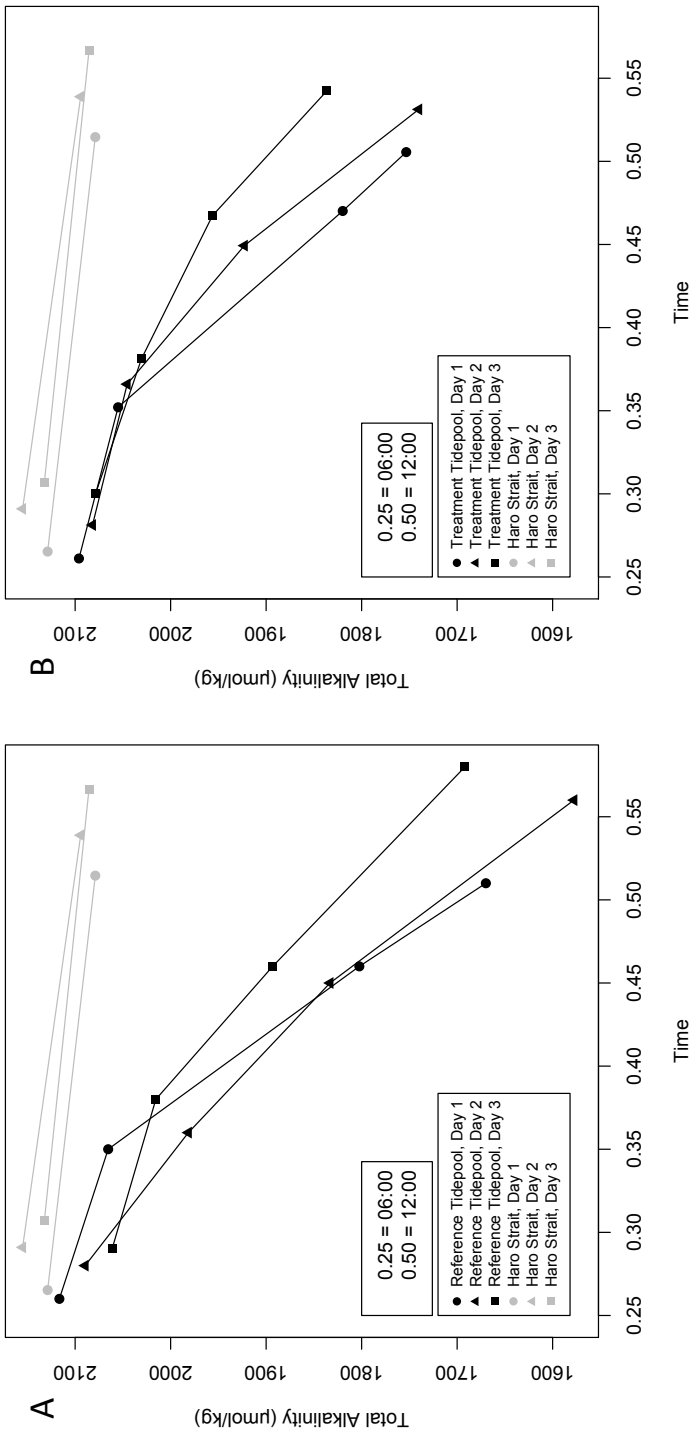

Figure 6: Total alkalinity for four sampling points for control day (Day 1), *Ulva* addition day (Day 2) and *Mytilus* addition day (Day 3) for the reference pool and Haro Strait (A) and the treatment pool and Haro Strait (B). For all days total alkalinity increased with time in both pools and the strait, with the sraight having a consistently less decrease than either of the pools. Time is expressed as a fraction of the day, where 0.25 corresponds to 6 am and 0.5 to 12 pm.

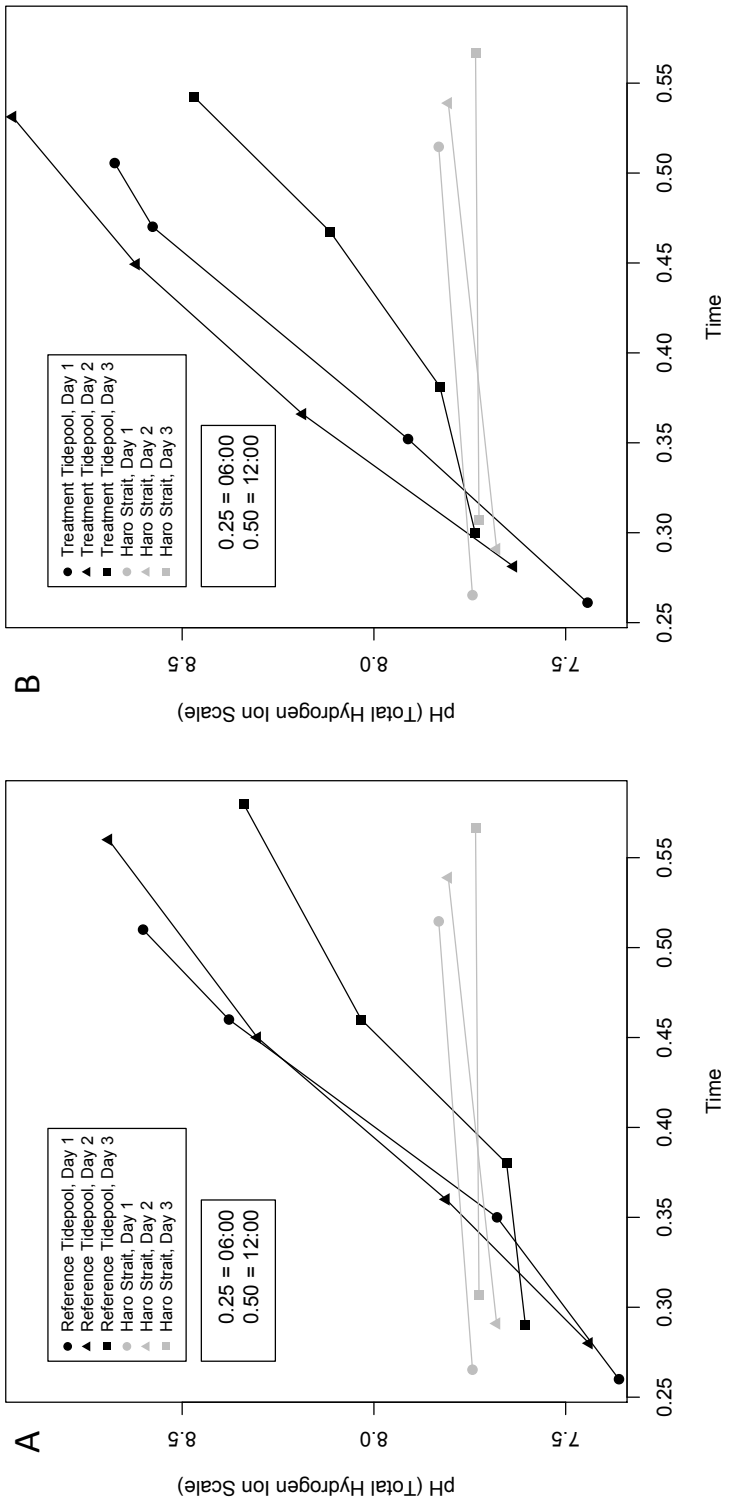

Figure 7: pH measured at the sample temperature at four different time points for control day (Day 1), *Ulva* addition day (Day 2) and *Mytilus* addition day (Day 3) for the reference pool and Haro Strait (A) and the treatment pool and Haro Strait (B). For all days pH increased with time in both pools and the strait. pH was consistently greater in the tidepools. Time is expressed as a fraction of the day, where 0.25 corresponds to 6 am and 0.5 to 12 pm.

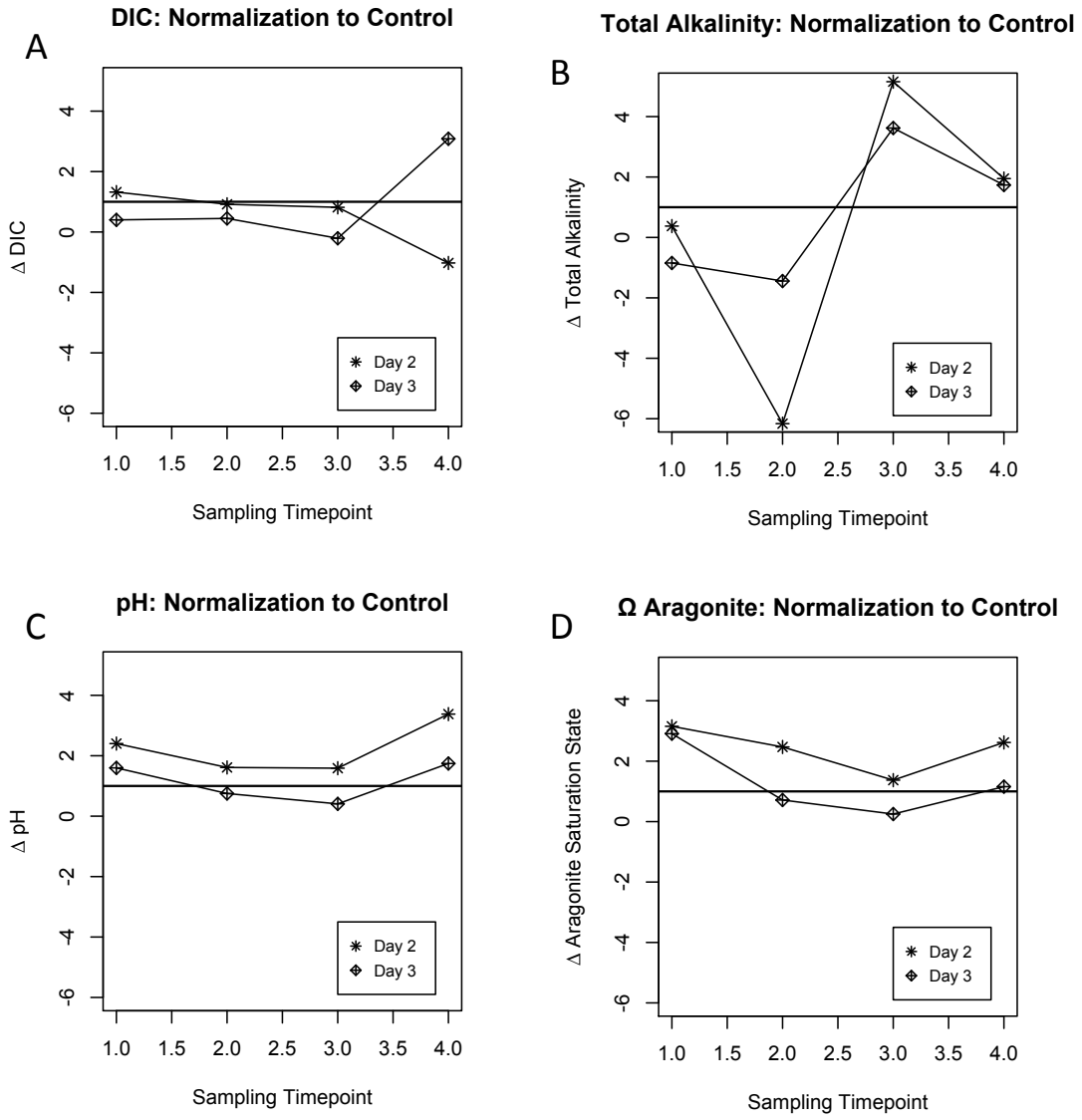

Figure 8: Normalization of DIC (A), Total Alkalinity (B), pH (C), and Ω Aragonite (D) of control day (Day 1), *Ulva* addition day (Day 2) and *Mytilus* addition day (Day 3) at the four sampling time points through the day. Values are normalized to the difference between the two tidepools at the corresponding time point on day 1, where a value of change in 1 corresponds to no difference between the reference pool and treatment pool

APPENDIX 1: *Ulva sp*. area determination methods

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 a oven (60ºC, 24hrs) and dry weights were obtained with an analytical balance. The remainder of the *Ulva sp*. used in this experiment was also dried in a oven (60˚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 of *Ulva sp*. added to the pool.

APPENDIX 2: Physical Parameters of each sample

Day	Sample ID	Location	Salinity	Temp	Alk	DIC	pH (25˚C)	pH (25˚C)	pH (25˚C)	pH(temp. adj.)	pCO2	ΩCa	Ω Arag	HCO_3^-
				(°C)	(µmol/kg)	(µmol/kg)	tb	mcp	CO2Calc	CO2Calc	(µatm)			(µmol/kg)
1	TP001	Reference	30.1	10.25	2116.53	2171.46	8.472	7.187	7.194	7.361	2088	0.623	0.392	2042.5
1	TP002	Treatment	30.8	10.10	2095.97	2125.43	8.561	7.290	7.266	7.443	1696	0.745	0.47	2008.2
1	TP003	Haro Strait	30.4	10.17	2128.81	2077.16	6.942	7.451	7.541	7.743	835.8	1.451	0.913	1969.0
1	TP004	Reference	30	11.32	2065.41	2028.15	8.578	7.446	7.499	7.68	952.8	1.275	0.803	1924.7
1	TP005	Treatment	30.3	10.98	2055.00	1951.53	7.461	7.662	7.709	7.911	532.2	2.05	1.292	1835.4
1	TP006	Reference	31.3	15.40	1802.41	1482.27	8.521	8.200	8.23	8.378	128.8	5.021	3.202	1268.0
1	TP007	Treatment	31.5	17.66	1819.80	1355.54	7.109	8.454	8.461	8.577	68.69	7.236	4.641	1055.6
1	TP008	Treatment	30.8	18.51	1753.29	1237.01	7.210	8.538	8.572	8.676	47.48	7.993	5.127	910.2
1	TP009	Reference	31.3	17.96	1669.86	1216.10	7.483	8.465	8.491	8.602	57.46	6.84	4.388	933.7
1	TP010	Haro Strait	30.1	12.25	2078.88	1994.65	7.061	7.625	7.651	7.831	663.4	1.831	1.156	1883.5
2	TP011	Reference	30	10.20	2089.51	2121.32	7.021	7.279	7.264	7.439	1714	0.729	0.458	2004.4
2	TP012	Treatment	30.1	10.15	2081.83	2060.52	7.222	7.409	7.443	7.636	1063	1.119	0.704	1955.7
2	TP013	Haro Strait	29.6	10.13	2154.99	2123.10	7.244	7.460	7.483	7.68	991.4	1.263	0.793	2015.7
2	TP014	Reference	30.1	11.39	1981.39	1908.33	7.138	7.610	7.622	7.812	659.2	1.626	1.025	1804.5
2	TP015	Treatment	30.2	11.24	2045.46	1838.12	7.236	7.949	7.977	8.186	258.3	3.549	2.238	1674.9
2	TP016	Reference	30.1	13.73	1833.05	1570.32	7.354	8.110	8.132	8.304	165.7	4.245	2.688	1386.0
2	TP017	Treatment	30.2	13.71	1922.78	1466.84	7.306	8.415	8.444	8.62	66.98	7.362	4.663	1161.3
2	TP018	Treatment	30.2	17.41	1740.23	1077.95	7.565	8.732	8.817	8.942	18.66	10.2	6.511	667.5
2	TP019	Reference	30.1	18.12	1577.71	1099.40	7.472	8.557	8.583	8.692	40.69	7.154	4.575	808.1
2	TP020	Haro Strait	30.1	11.27	2094.15	2021.23	7.245	7.602	7.616	7.806	707.9	1.691	1.066	1912.0
3	TP021	Reference	30.4	10.40	2061.31	2046.31	7.858	7.354	7.418	7.605	1133	1.052	0.662	1942.4
3	TP022	Treatment	30.8	10.27	2078.66	2027.87	7.786	7.510	7.535	7.736	828.5	1.409	0.889	1922.7
3	TP023	Haro Strait	30.8	10.25	2132.20	2083.68	n/a	7.481	7.526	7.726	870.6	1.416	0.892	1976.1
3	TP024	Reference	30.8	11.00	2015.50	1984.35	n/a	7.413	7.47	7.815	623.4	1.104	0.687	1883.4
3	TP025	Treatment	31	11.10	2030.51	1949.97	8.281	7.589	7.632	7.998	399.7	1.651	1.028	1841.9
3	TP026	Reference	30.7	12.00	1893.15	1747.80	7.648	7.757	7.841	8.222	210.1	2.423	1.508	1622.7
3	TP027	Treatment	30.9	12.20	1954.01	1774.20	8.164	7.856	7.918	8.304	174.8	2.948	1.835	1628.7
3	TP028	Treatment	30.8	13.80	1836.44	1479.05	7.278	8.244	8.295	8.694	54.15	5.507	3.427	1240.2
3	TP029	Reference	30.6	14.70	1691.79	1414.52	8.787	8.141	8.182	8.577	70.46	4.151	2.582	1230.1
3	TP030	Haro Strait	30.8	11.30	2085.47	2030.70	8.247	7.580	7.548	7.904	518.9	1.384	0.862	1924.9