

Effects of Ocean Acidification on Marine Calcium Carbonate

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SUMMARY

Ocean acidification poses a serious risk to marine organisms with calcium carbonate exoskeletons. The purpose of this study was to investigate the effects of acidification on the mass of corals and shells composed of calcium carbonate. Corals and shells composed of calcium carbonate were placed in four sets of jars with different pH levels. After six weeks, we measured the change and percentage change in mass of the corals and shells. Our results indicated that there was no significant difference in the percent change in mass for the corals and shells exposed to different pH levels. However, the corals experienced significantly greater loss of mass than the shells exposed to the same experimental conditions. This suggests that ocean acidification may pose a greater risk to corals than it does to shells. Given the role played by corals in the marine ecosystem, these findings emphasize an urgent need for mitigating and reversing the effects of ocean acidification.

INTRODUCTION

Since the Industrial Revolution, the amount of carbon dioxide in the atmosphere has been increasing (1). Because the oceans absorb approximately one quarter of fossil fuel-related carbon dioxide emissions, there has been a corresponding increase in the absorption of carbon dioxide by the oceans (2, 3). This leads to a decrease in the pH of seawater, resulting in a phenomenon known as ocean acidification (4). In the last 200 years, there has been a 30% increase in ocean acidity (1). The current pH of the ocean is approximately 8.1, whereas in 1800, it was estimated to be approximately 8.2 (5, 6). As oceans absorb carbon dioxide, dissolved inorganic carbon molecules buffer changes in the pH level of the ocean. These dissolved inorganic carbon molecules are provided, in part, by dissolution of the carbonate shells of marine organisms. However, the recent rapid increase in carbon dioxide overwhelms the natural buffering capacity of the ocean, resulting in a rapid drop in the pH of the world's oceans (7).

Ocean acidification constitutes a major problem to marine life, in particular to organisms with calcium carbonate exoskeletons. Carbonate ions and calcium are the materials that hard-shelled organisms use to make their shells (3). When carbon dioxide is absorbed by the oceans, carbonic acid is formed through the following reaction: CO_2 (carbon dioxide) + H_2O (water) \leftrightarrow H_2CO_3 (carbonic acid) (8). Some of the carbonic acid dissociates into hydrogen

ions and bicarbonate as follows: $\text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+$ (hydrogen ions) + HCO_3^- (bicarbonate). The hydrogen ions react with carbonate ions (CO_3^{2-}) found in the oceans to create more bicarbonate, which removes carbonate ions from the water. Thus, increasing acidification results in the conversion of carbonate ions into bicarbonate, which cannot be utilized for calcium carbonate formation by marine organisms. If there is a decreased supply of carbonate ions, then organisms such as corals, oysters, clams, and mussels will find it harder to survive. In addition, continued acidification of the oceans could result in dissolution of already formed calcium carbonate (3). Due in part to the effects of ocean acidification, many coral reefs have been disappearing (3). Besides corals, oysters, clams and mussels, many other marine organisms utilize calcium carbonate for their exoskeletons. For instance, coccolithophores, unicellular protists surrounded by shells composed of calcium carbonate, are also significantly impacted by ocean acidification. Coccolithophores are at the base of the marine food web, and they play a large role in recycling nutrients and droppings throughout the ocean (5). Thus, ocean acidification can have far reaching consequences to both marine life and the humans that depend on it.

In summary, ocean acidification affects marine organisms with calcium carbonate exoskeletons in two ways. First, the acidity in the ocean can dissolve shells composed of calcium carbonate. Second, ocean acidification shifts the pH balance of the ocean toward the generation of bicarbonate ions, which marine organisms cannot utilize to make their shells. In this study, we aimed to investigate the effects of increased acidity on pre-existing calcium carbonate found in corals and shells. Because calcium carbonate dissolves in more acidic solutions, we tested the hypothesis that corals and shells exposed to seawater with the lowest pH would lose the greatest percentage of their mass after six weeks of exposure.

RESULTS

Change in Mass over the Course of the Experiment.

To test the effects of acidity on shells and corals, we exposed the shells and corals to simulated seawater with different pH conditions of 8.6, 8.1, 7.6, and 7.1, adjusting the pH to baseline every week. At the end of six weeks, we determined if the weights of the shells and corals in the different experimental conditions changed from baseline. After six weeks of exposure (Table 1 and Figure 1), the shells in the control group (pH 8.6) lost on average 0.27 (SD \pm 0.09)

pH level	Jar ID	Shell Mass			Coral Mass		
		Before (g)	After (g)	% change	Before (g)	After (g)	% change
8.6	A1	2.606	2.599	0.27	6.403	6.361	0.66
	A2	2.606	2.601	0.19	6.84	6.788	0.76
	A3	2.776	2.771	0.18	6.489	6.443	0.71
	A4	2.875	2.866	0.31	7.748	7.690	0.75
	A5	2.720	2.709	0.40	7.697	7.587	1.43
8.1	B6	2.771	2.765	0.22	7.738	7.690	0.62
	B7	2.528	2.526	0.08	6.655	6.575	1.20
	B8	2.637	2.634	0.11	8.126	8.067	0.73
	B9	2.511	2.508	0.12	7.466	7.384	1.10
	B10	2.707	2.701	0.22	6.684	6.608	1.14
7.6	C11	2.614	2.604	0.38	8.804	8.745	0.67
	C12	2.824	2.819	0.18	8.267	8.185	0.99
	C13	2.830	2.826	0.14	8.525	8.455	0.82
	C14	2.601	2.595	0.23	7.621	7.562	0.77
	C15	2.849	2.843	0.21	7.177	7.130	0.65
7.1	D16	2.681	2.666	0.56	5.958	5.899	0.99
	D17	2.739	2.732	0.26	9.826	9.734	0.94
	D18	2.577	2.572	0.19	6.021	5.961	1.00
	D19	2.702	2.690	0.44	7.422	7.267	2.09
	D20	2.681	2.671	0.37	6.342	6.294	0.76

Table 1. Individual Change in Mass in Shells and Corals. This table shows the mass of the shells and corals before and after exposure to simulated seawater with varying levels of acidity and the percent change in mass (calculated by dividing the change in mass by the original mass). Jars A1-A5 were control jars, with pH of 8.6, jars B6-B10 had simulated seawater with pH of 8.1, jars C11-C15 had simulated seawater with pH of 7.6 and jars D16-20 had simulated seawater with pH of 7.1.

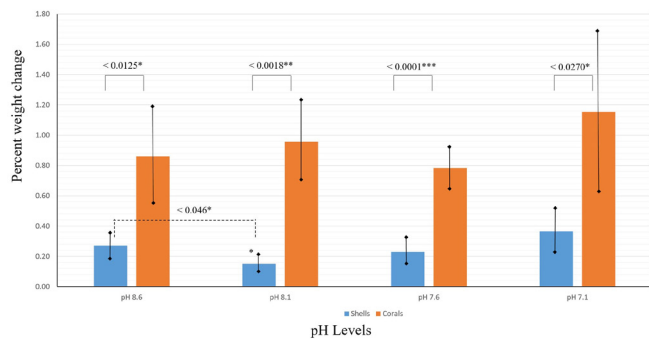


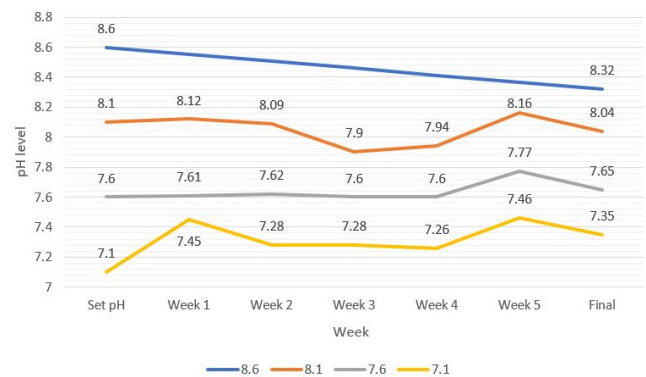
Figure 1. Average Loss in Mass in Shells and Corals. This graph shows the average percent loss of mass for the shells and corals in each experimental condition. The error bars depict the standard deviation. For each pH level, the percentage loss of mass was significantly different between the shells and corals. P-values are shown. When the shells and corals in the control group (pH 8.6) were compared to the shells and corals in the experimental groups, the only statistically significant difference was that between the shells in the first experimental group (pH 8.1) and the control ($p < 0.046$), denoted by the dashed line.

% of their mass. The shells in the first experimental group (pH 8.1) lost on average 0.15 (SD \pm 0.06) % of their mass ($p=0.046$ vs control). The shells in the second experimental group (pH 7.6) lost on average 0.23 (SD \pm 0.09) % of their mass, while the shells in the third experimental group (pH 7.1) lost on average 0.37 (SD \pm 0.15) % of their mass. The differences in percent loss of mass among the shells in the second and third experimental groups compared to the control group were not statistically significant.

When looking at the corals, after six weeks of exposure (Table 1 and Figure 1), the control group (pH 8.6) lost on average 0.86 (SD \pm 0.32) % of their mass. The corals in the first experimental group (pH 8.1) lost on average 0.96 (SD \pm

pH	Shells		Corals		P-Value Shells v Corals
	Mass Change (% \pm SD)	P Value	Mass Change (% \pm SD)	P Value	
8.6	0.27 \pm 0.09	-	0.86 \pm 0.32	-	0.0125
8.1	0.15 \pm 0.06*	.046*	0.96 \pm 0.26	.62	0.0018
7.6	0.23 \pm 0.09	.48	0.78 \pm 0.14	.64	0.0001
7.1	0.37 \pm 0.15	.27	1.15 \pm 0.53	.33	0.0270
All	0.25 \pm 0.12	-	0.94 \pm 0.35	-	1.67 $\times 10^{-8}$

Table 2. Average Percent Change in Mass for Shells and Corals. This table shows the average percent change in mass of the shells and corals for the varying pH conditions. In column 3, results of comparisons between the percent loss of mass of the shells in the control jars (pH 8.6) versus the experimental groups are shown. In column 5, results of comparisons between the percent loss of mass of the corals in the control jars (pH 8.6) versus the experimental groups are shown. Column 6 depicts the results of comparisons between the percent loss of mass of the shells versus corals for each of the pH conditions as well as for the shells in all 20 jars versus the corals in all 20 jars. All p-values were calculated using t-tests (Microsoft Excel).



The values of the pH levels of the simulated seawater throughout the experiment are shown. The pH levels of the jars of the experimental groups (pH 8.1, 7.6, and 7.1) were adjusted to the intended target pH every week. The pH levels of the control jars (pH 8.6) were not measured weekly.

0.26) % of their mass. The corals in the second experimental group (pH 7.6) lost on average 0.78 (SD \pm 0.14) % of their mass, and the corals in the third experimental group (pH 7.1) lost on average 1.15 (SD \pm 0.53) % of their mass. The differences in percent loss of mass among the corals in each of the experimental groups versus the control group were not statistically significant.

After six weeks of exposure, the percent loss of mass between the shells and corals within each experimental group was compared (Table 2 and Figure 1). At a pH of 8.6, the percentage loss of mass of the shells was significantly lower than that of the corals (0.27 \pm 0.09 vs 0.86 \pm 0.32; $p < 0.0125$). At a pH of 8.1, the percentage loss of mass of the shells was significantly lower than that of the corals (0.15 \pm 0.06 vs 0.96 \pm 0.26; $p < 0.0018$). At a pH of 7.6, the percentage loss of mass of the shells was significantly lower than that of the corals (0.23 \pm 0.09 vs 0.78 \pm 0.14; $p < 0.0001$). At a pH of 7.1, the percentage loss of mass of the shells was significantly lower than that of the corals (0.37 \pm 0.15 vs 1.15 \pm 0.53; $p < 0.0270$). Finally, when shells were compared to corals at all pH levels, the percentage loss of mass of the shells was significantly

less than that of the corals (0.25 ± 0.12 vs 0.94 ± 0.35 ; $p < 1.67 \times 10^{-8}$).

Change in pH over the Course of the Experiment.

Since dissolved calcium carbonate could potentially have buffered the pH of the simulated seawater, we measured the pH levels in the experimental groups and adjusted them to baseline as necessary. The pH of the jars in experimental group 1 (target 8.1; orange line) decreased about 0.1 every week, reaching 7.86 in week 3 (**Figure 2**). At the end of the observation period, the pH was 8.04. The pH of the jars in experimental group 2 (target 7.6; grey line) did not vary much; the pH was relatively stable and only had to be adjusted in week 5 when it was 7.77. The pH at the end of the study period averaged to 7.65. The pH of the jars in experimental group 3 (target 7.1; yellow line) became alkaline every week, reaching an average of 7.46 in week 5. At the end of the observation period, the pH was 7.35. The control jars were not examined weekly and were left undisturbed. At the end of study period, the pH had dropped to 8.32 from 8.6 at baseline.

DISCUSSION

Our results indicate that corals and shells placed in jars of simulated seawater with different levels of acidity experienced loss in mass after six weeks in all conditions, an effect which was more pronounced in the corals than in the shells. The differences in percent loss of mass among the shells in the second and third experimental groups versus the control group were not statistically significant (**Figure 1**). Although there was a statistically significant difference in the percentage of loss of mass between the control group and the first experimental group (pH 8.1), the shells exposed to a lower pH actually lost less mass, suggesting that this was due to a Type I statistical error, which is associated with false positive results, reflecting the number of comparisons in the experiment. Though the corals exposed to a pH of 7.1 and 8.1 lost more mass than those in the control group, these differences were not statistically significant. Thus, this experiment did not support the primary hypothesis that shells and corals exposed to a pH of 7.1 would lose the most mass.

However, the results demonstrate that there was a clear difference in the dissolution rates between the corals and the shells exposed to the same experimental conditions. For each pH condition, the corals had a higher percent loss of mass compared to the shells. Furthermore, when we compared the percent loss of mass of the shells and corals in all 20 jars, we found that the difference between corals and shells was highly statistically significant (0.25 ± 0.12 vs 0.94 ± 0.35 ; $p < 1.67 \times 10^{-8}$).

The magnitude of loss in mass among the corals was greater than that of the shells. This result could be due in part to the type of calcium carbonate in these structures. Corals are composed of aragonite, whereas shells are composed of calcite (5). The results of this experiment, then, suggest that aragonite may be more susceptible to the

effects of ocean acidification. Hence, if ocean acidification progresses at its current rate, we speculate that it will have a more significant effect on marine life, especially corals and other marine organisms that have exoskeletons composed of aragonite. The corals that were exposed to water with a pH of 7.1 lost more than 1% of their mass after merely six weeks of exposure. If the demineralization continued at or near this rate, extrapolation predicts that almost ten percent of the mass would be lost in a year. Coral polyps must create new exoskeletons to replace the demineralized ones, which limits their ability to survive and reproduce. Additionally, it has been shown that different species of corals differ in their susceptibility to the effects of acidification and thermal stress (10). Therefore, increases in ocean acidification could result in alteration of the types of coral communities, consequently affecting the marine ecosystem. Coral reefs are an essential habitat to millions of marine species, so alterations in their structure could lead to an unbalanced ecosystem, which would also significantly impact the food web.

The pH of the water in the experimental jars fluctuated throughout the experiment, and this fluctuation was most notable in the 7.1 pH experimental group. This is likely due to the dissolution of the calcium carbonate resulting in neutralization of the acidity, requiring weekly addition of acetic acid to reset the pH to the target of 7.1. The other experimental groups fluctuated much less. We speculate that this could reflect a lower rate of dissolution at the other pH levels.

This experiment does have limitations. Firstly, the pH levels of the jars in the experimental groups were checked weekly and adjusted as necessary. This could have introduced experimental error because not all of the jars required adjustment in pH every week. Additionally, shells and corals in the ocean would be surrounded by extremely large volumes of water and would be subjected to ocean currents. Therefore, the oceanic pH would likely not have been influenced by the dissolution of marine calcium carbonate as observed in the relatively small jars that were used in this experiment. A much larger tank would have provided a more ocean-like setting than the current setup. Another variation to this experiment could have been to use real seawater rather than simulated seawater. With real seawater, there would be additional minerals and salts that were not accounted for in this experiment. Additionally, if the duration of exposure had been longer, there likely would have been more change in the mass of the shells and corals. Finally, carbon dioxide could have been used instead of acetic acid solution, to more closely mirror the actual process of ocean acidification. However, the accurate regulation of the amount of carbon dioxide would have necessitated the acquisition of expensive equipment called a flow controller, which was beyond the scope of this experiment. Since the addition of acetic acid to seawater results in the release of carbon dioxide, use of acetic acid is a good proxy for studying ocean acidification.

This experiment validates the harmful effects of ocean

acidification on marine life and suggests that ocean acidification is particularly harmful to corals. Coral reefs are estimated to shelter more than a quarter of all marine species (11). Consequently, a loss of corals would significantly endanger many aquatic organisms, potentially causing their extinction. Furthermore, reefs and atolls, which are composed of corals, are also at risk. For instance, Kiritimati Atoll, also known as Christmas Atoll, is the largest coral atoll in the world. It has a population of 6,000 people and has been a crucial refueling center for aircraft (12). If the coral atoll were to dissolve, then the people would have to evacuate and change their lifestyle. Additionally, industries, such as the seafood industry, are also impacted by ocean acidification. In the Pacific Northwest, for example, ocean acidification is already a challenge to the 100-million-dollar oyster industry (13). Due to a shortage of oysters, as many as a thousand jobs have been jeopardized. Other industries will similarly be impacted economically by ocean acidification.

In conclusion, marine organisms with calcium carbonate exoskeletons play a crucial role in the ocean ecosystem. The detrimental effects of ocean acidification on corals, as suggested by our work, could have devastating consequences that alter the way of life for countless species of marine organisms, as well as the humans who depend on them.

MATERIALS AND METHODS

A Homgeek professional digital mini-scale TL and a TaDaKool pH meter were calibrated according to the manufacturer's instructions. Twenty dry 473-milliliter mason jars (Ball Pint Jars) were labeled, and each jar held 3 white cardium cockle shells (*Cerastoderma edule*) and 1 tiny white mushroom coral (*Lobactis scrutaria*) of pre-determined mass. The shells and corals were obtained from Richard's Seashells, Winter Haven, FL (deltonaseashells.com). Ten liters of distilled water were poured into a bucket and allowed to equilibrate. A sufficient quantity of Instant Ocean® Sea Salt for Marine Aquariums (0.36 liters) was added to the water to bring it to a pH of 8.6. Then, jars 1-5 were filled to the brim with this simulated seawater, and the lids were sealed tightly. These were the control jars. A diluted solution (0.6% concentration) of acetic acid was created using 30% white vinegar, manufactured by Energen of Carolina. Then, the diluted solution was carefully added to the bucket using a dropper until the pH measured 8.1 and then was mixed well. Jars 6-10 were filled as described above. This was experimental group 1. Similarly, additional quantities of diluted acetic acid solution were added to yield simulated seawater with pH values of 7.6 and 7.1, which were used to fill jars 11-15 and 16-20, respectively. These were experimental groups 2 and 3.

Once a week, the jars in the experimental groups were opened and the pH levels were measured. If necessary, diluted acetic acid solution or TopFin pH increase, which is a solution of sodium bicarbonate, was added to each jar in order to reset the target pH. The control jars were not opened.

Once finished, the lids were screwed on tightly and set aside. At the end of the six week period, all jars were emptied of the water. The shells and corals were washed gently with distilled water and allowed to dry on paper towels. After four weeks of drying, the mass of the shells and corals were measured and recorded. The percent change in mass (calculated by dividing the change in mass by the original mass) and standard deviation (SD) were then calculated for each group separately for shells and corals. The percent changes in mass between the control and the experimental groups were compared using two-tailed t-tests (using Microsoft Excel) separately for shells and corals. Additionally, for each pH value, the percent changes in mass between the shells and corals were computed using two-tailed t-tests. Lastly, a two-tailed t-test was used to compare the percent changes in mass between all shells and all corals.

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