Journal of
Emerging Investigators

Effect of pH change on exoskeletons of selected saltwater organisms which rely on calcium fixation

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Summary

The projections for rising atmospheric carbon dioxide concentrations indicate that the pH levels of the ocean surface could decrease by 0.3-0.4 units by the end of the 21st century. The objective of this research was to evaluate the effect of pH on the exoskeletons of six aquatic organisms commonly found in South Florida coastal waters. The exoskeleton samples studied were from the common nutmeg (*Cancellaria reticulate*), lettered olive (*Oliva sayana*), stiff pen shell (*Atrina rigida*), kitten's paw (*Plicatulidae*), fan coral (*Gorgonia ventalina*), and common slipper shell (*Crepidula fornicate*). The exoskeleton samples were exposed to saltwater (34% salinity) at pH levels ranging from 8.3 to 6.0 for 5 days. The changes in the masses of the samples before and after exposure were compared. We normalized the data in reference to the observations at pH=8.3, which is the current pH level of the ocean surface. The fan coral had the highest percent mass loss, which increased with decreasing pH until pH 6.5. Exoskeleton samples from the stiff pen shell were not affected by the pH changes. Exoskeletons from the common nutmeg, lettered olive, and common slipper shell had similar responses to pH changes with relatively small changes in mass. Exoskeletons from the kitten's paw had significant mass loss at lower pH conditions. The research results show that as ocean acidification increases, the exoskeletons of marine organisms will be affected. Some organisms, such as the fan coral and kitten's paw, may lose their exoskeletons. Other organisms that rely on the marine organisms with exoskeletons for shelter and food could also be affected.

Received: Jul 12, 2013; **Accepted:** Oct 31, 2013; **Published:** Jan 2, 2014

Introduction

Analyses of the chemical changes associated with historical carbon dioxide (CO_2) emissions indicate that the average pH of the ocean surface has decreased by about 0.1 units, which is equivalent to about a 25% increase in H^+ concentration in the last 200 years (1,2,3). According to atmospheric CO_2 projections, ocean surface pH levels are estimated to decrease by 0.3-0.4 units by the end of the 21st century. This decrease corresponds to an increase in the hydrogen ion concentration of about 100-150% above the levels in the late 1800s (4,5). The impacts of ocean acidification can be 10–50% higher near coastal areas due to proximity to anthropogenic sources (6).

Although some species can tolerate pH changes, many marine organisms and processes can be impacted, including the composition of communities and food webs (7). Studies show that ocean acidification will have direct impacts on the calcification and the growth of stony corals (4, 8). Experiments conducted on large benthic foraminifers (major contributors to organic and inorganic carbon production in coral reefs) indicate that the growth rate (measured by shell diameter, shell weight, and number of chambers added) generally decreases with lower pH after 10 weeks and that the shell weight is the most closely dependent parameter to pH (9). Around pH 7.7, the calcification rate in benthic organisms declines significantly.

Calcium carbonate has three crystal polymorphs: calcite, aragonite, and vaterite (in order of decreasing thermodynamic stability) (10). Calcifying aquatic organisms do not necessarily form the most stable calcites but most often form the relatively less stable aragonite and vaterite. Crustaceans usually deposit calcium carbonate in amorphous forms as aragonite and vaterite (11,12,13). The existence and role of more than one amorphous form of calcium carbonate in biominerals have only been understood very recently (14). The exoskeletons of these aquatic organisms contain phospenolpyruvate (PEP) and 3-phosphoglycerate (3PG), which may inhibit the crystallization of calcium carbonate in a denser packing structure, thus leading to the formation of amorphous forms of calcium carbonate as aragonite and vaterite (15). The changes in packing to denser forms during biomineralization provide protection during periods of dormancy, when the rates of some of the metabolic processes are reduced (14, 16). The presence of phosphorus may also increase the stability of calcified exoskeletons (14,17).

The objective of this research was to evaluate the effect of pH on the exoskeletons of six aquatic organisms

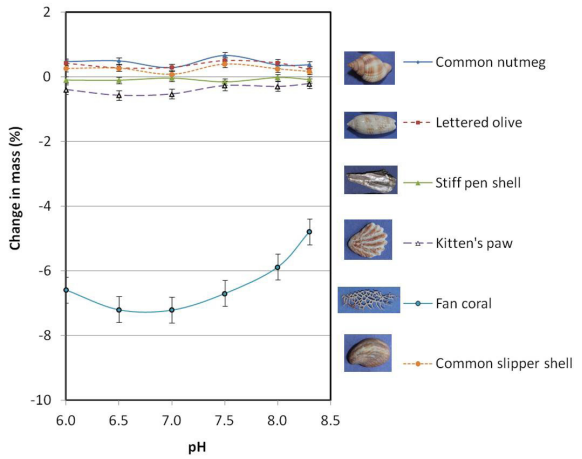

Figure 1: Change in the mass of exoskeletons exposed to saltwater at different pH levels.

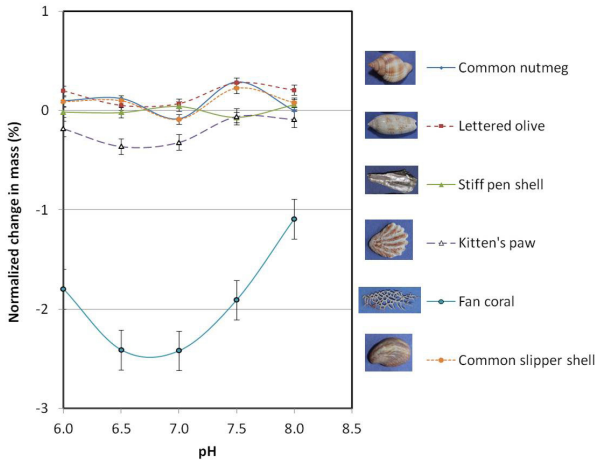

Figure 2: Change in exoskeleton mass in reference to change observed at pH=8.3.

found in Miami, FL, USA. We exposed the exoskeletons from six organisms to saltwater at pH levels ranging from 8.3 (current ocean surface pH) to 6.0. The effect of pH was evaluated based on the percent change in mass after a 5-day exposure.

Results

We compared the exoskeletons from six aquatic organisms to pH changes. These were the common nutmeg (*Cancellaria reticulate*), lettered olive (*Oliva sayana*), stiff pen shell (*Atrina rigida*), kitten's paw (*Plicatulidae*), fan coral (*Gorgonia ventalina*) and common slipper shell (*Crepidula fornicate*). After a 5-day exposure, the exoskeletons showed different responses (measured as a percent change in mass) to saltwater at pH levels ranging from 8.3 (current ocean level) to 6.0 (**Figure 1**).

We normalized the data with respect to percent mass change observed at pH 8.3 to evaluate the effects of lower pH levels in relation to the current pH of the ocean. Figure 2 presents the normalized percent change in the mass of exoskeleton samples. The normalized percent mass loss for fan coral was the highest at all pH levels. The normalized mass loss for fan coral at pH 8.0 and 7.5 were -1.09±0.05% and -1.91±0.05%, respectively. The kitten's paw had the second highest mass loss trend with a normalized mass loss of -0.10±0.05% at pH 8.0. Although there were some changes in the masses of the exoskeleton samples from the common nutmeg, lettered olive, and common slipper shell, there were no clear trends with decreasing pH. The exoskeleton samples from the stiff pen shell were the most resistant to pH changes with no significant mass change observed at any pH level.

The results showed that even for relatively short exposure periods (5 days), the exoskeletons of aquatic organisms can be affected. As the ocean acidification increases, organisms such as fan coral and kitten's paw may lose their exoskeletons and may even disappear. The potential effects on the exoskeletons of the species studied for the case when the ocean pH is reduced from 8.3 to 7.5 are summarized in Figure 3.

Discussion

Biomineralization processes, by which aquatic organisms are able to precipitate minerals, involve complex interactions. We determined whether exoskeletons would dissolve by exposing them to saltwater at different pH levels. The exoskeleton samples that were exposed to saltwater buffered at different pH levels showed very different responses in terms of percent change in mass. Due to the mass increases observed for some samples, the data were normalized to the change in mass at pH 8.3, which is the pH level of the ocean surface where the samples were collected. Some of the samples showed an increase in mass at lower pH levels. This increase may be due to buffering effects and changes in the ionic balances of the solutions which can change in the hydration characteristics and composition of the exoskeleton biominerals (i.e., formation of hydrated minerals or precipitation of HCO_3^- on the samples) at lower pH conditions.

We observed the following results in our experiments:

1. The exoskeletons from fan coral and the kitten's paw had mass losses at all pH levels studied (pH=6.0-8.3).

2. The fan coral had the highest mass loss at all pH levels.

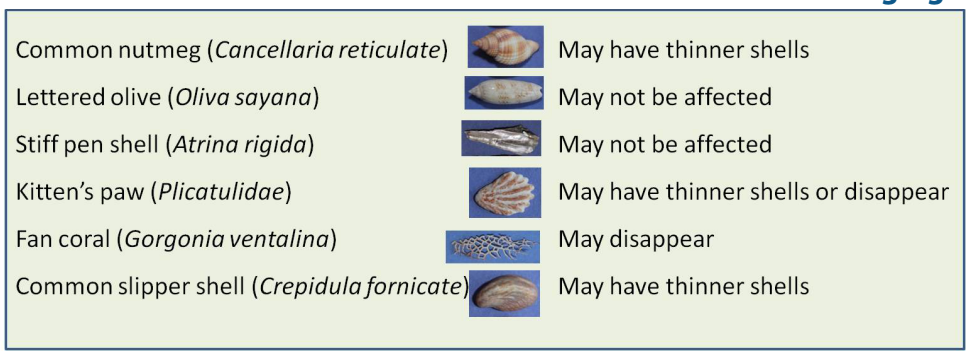

Figure 3: Potential effects on exoskeletons of the species studied if ocean pH is reduced from 8.3 to 7.5.

3. The exoskeleton from the stiff pen shell had no noticeable change in mass.

4. Exoskeleton samples from the common nutmeg, lettered olive, and common slipper shell did not have significant changes in mass at lower pH conditions in reference to those observed at pH 8.3.

These findings could have the following implications:

1. A change in the pH level of the oceans could affect the exoskeletons of some of the aquatic organisms.

2. Some organisms, such as the fan coral and kitten's paw, are likely to experience a loss in the mass of their exoskeletons as the ocean pH decreases.

3. Other marine organisms that rely on the organisms with exoskeletons for shelter and food could also be affected.

In most biological systems, the mineral deposition site is isolated from the environment by the geometric shape of the organism (e.g., compartment). The ability of the organisms to form an enclosure provides limited diffusion into and out of the system. This enclosure allows the conditions for modifying the activity of at least one biomineral constituent (i.e., cation) as well as protons and possibly other ions. However, due to increasing ocean acidification some organisms, such as corals and species which cannot compartmentalize water to limit ion transfer, could to be impacted due to the loss of or difficulty in maintaining their exoskeletons.

This research shows that decreasing pH does not necessarily decrease the mass of the exoskeletons of all organisms. However, it prompts other questions that need to be explored. The biomineralization mechanisms of aquatic organisms depend on ionic balances, temperature, and pressure as well as genetically controlled mechanisms. The combined effects of changes in water temperature and pH could result in shifts in the aquatic ecosystems that affect the diversity of the species in the oceans.

Methods

Materials

We conducted the experiments with 34% saltwater, which is the typical salinity of ocean water. We prepared the saltwater with Instant Ocean Sea Salt (Aquarium System, Mentor, Ohio, USA) and deionized water. We prepared the phosphate buffer solutions for the pH range 6.0–8.0 at 25°C, according to the procedure described in Standard Methods for the Examination of Water and Wastewater (18). We chose the lowest pH level as 6 because CO_3^{-2} ions are present only above pH 6 in aqueous systems. Below pH 6, the equilibrium favors the presence of only HCO_3^- ions and H_2CO_3 (8). We wanted the presence of CO_3^{-2} ions in the seawater so that calcium carbonate could exist in solid state. With decreasing CO_3^{-2} and increasing HCO_3^- levels, the seawater becomes increasingly undersaturated for calcium carbonate (i.e., aragonite and vaterite) .We prepared the solutions at different pH levels by placing 100 mL saltwater into Erlenmeyer flasks and added the

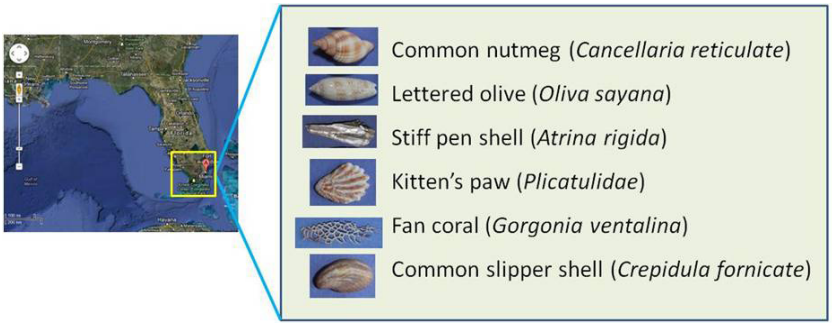

Figure 4: Exoskeleton samples collected from South Florida coastal areas.

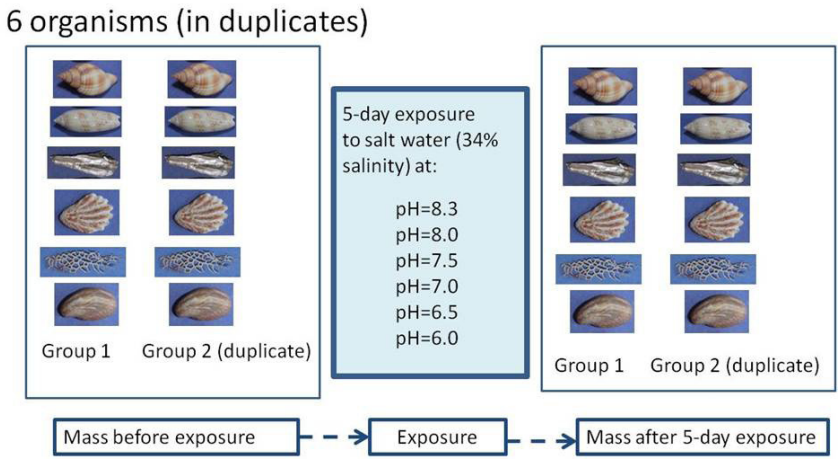

Figure 5: Procedural steps for the exposure experiments.

buffer solutions slowly (0.5-1.0 mL) until the desired pH. The prepared saltwater was at pH 8.3 and no buffering was needed.

Exposure procedure

The six exoskeleton samples investigated included commonly found shells and coral pieces in South Florida beaches in Miami area. These were from the common nutmeg (*Cancellaria reticulate*), lettered olive (Oliva sayana), stiff pen shell (*Atrina rigida*), kitten's paw (*Plicatulidae*), fan coral (*Gorgonia ventalina*), and common slipper shell (*Crepidula fornicata*) as presented in Figure 4.

Figure 5 illustrates the general steps for the exposure experiments. The exposure experiments were conducted in 50 mL test tubes with caps (72 test tubes, 36 of which are for duplicates). After pH adjustments, 20 mL of saltwater was added into the test tubes. The exoskeleton samples were first weighted and gently placed into the test tubes. The test tubes were capped to prevent water loss by evaporation. After 5 days, the exoskeleton samples were removed and placed on a tray that was covered with aluminum foil (with sides open) to dry. After 5 days, the samples were weighed and visually examined. Table 1 presents the variables that were controlled for and monitored during the exposure experiments. We averaged the measurements from the duplicate tests. We calculated the percent change in mass by the following equation:

Variable	Factors
Controlled variables	Salinity of water Volume of water Exposure time
Independent variables	Exoskeletons from different aquatic organisms pH of saltwater solutions
Dependent variable	Change in weight of exoskeletons (% w/w)

Table 1: Controlled and monitored variables.

$C_{pH} = ((W_b – W_a) / W_b) \times 100$

where,

C_{pH} : weight change (%)

W_b : weight of sample before exposure (g)

W_a : weight of sample after exposure (g)

We normalized the weight change observed at the pH levels studied in relation to the weight change observed at pH 8.3 as follows:

$C_{NpH} = C_{pH} – C_{8.3}$

where,

C_{NpH} : normalized weight change at the pH level studied (%)

C_{pH} : : weight change at the pH level studied (%)

$C_{8.3}$: weight change at pH=8.3 (%)

Editor's Note

Higher resolution images are available online at: http://www.emerginginvestigators.org

Acknowledgments

Mr. Eric Molina, biology teacher at Coral Gables Senior High in Miami, Florida, was the supervisor of the project and contributed to the improvement of the manuscript. The experiments were performed in the Water Quality Laboratory in the Environmental Engineering Department at Florida International University, Miami, Florida. The buffer solutions were prepared with assistance from Dr. Anna Bernardo Bricker.

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