

# Effects of Ocean Acidification on Limpet *Lottia scutum*'s Shell Strength

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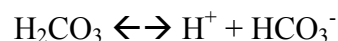
Key Words: Limpet, *Lottia scutum*, Ocean acidification

## Abstract

Calcium carbonate shells undergo dissolution in acidified conditions. As carbon dioxide in the atmosphere increases, the ocean's pH is lowered. The limpet *Lottia scutum* is used in this experiment to demonstrate the affect of ocean acidification on calcium carbonate material, which many marine organisms possess. As a limpet's shell becomes brittle its life expectancy may become threatened by environmental conditions such as desiccation, sun and temperature exposure, as well as predation. Peak force from crushing limpet shells is used in this experiment to measure shell strength and show how strength is diminished in acidified water over time.

## Introduction

Ocean acidification is a new and important concern for marine scientists as organisms are threatened by damage and calcium carbonate containing animals cannot produce shell as efficiently. As increasing amounts of carbon dioxide (CO<sub>2</sub>) enter the atmosphere, increasing amounts of CO<sub>2</sub> enter the ocean and lower its pH (Orr et al. 2005). Carbon dioxide constantly dissolves into the ocean and is kept at equilibrium, but as more CO<sub>2</sub> enters the ocean, its pH will gradually be lowered (Logan 2010). Throughout the past few decades, growing quantities of the atmosphere's carbon dioxide dissolve into the ocean annually (Feely 2004). As CO<sub>2</sub> dissolves in the ocean, it reacts to form carbonic acid (H<sub>2</sub>CO<sub>3</sub><sup>-</sup>), which disassociates into bicarbonate (HCO<sub>3</sub><sup>-</sup>) and hydrogen ions (H<sup>+</sup>), which lower the ocean's pH:



An acidified environment should slow the production and secretion of calcium carbonate shells in marine organisms. As hydrogen ion concentrations increase, it

becomes harder for calcifying organisms to build calcium carbonate shells (Orr et al. 2005). Ocean acidification should cause increased dissolution of calcium carbonate material and may have a negative impact on the overall growth rate of marine mollusks and other groups (Nienhuis 2004).

Large numbers of marine invertebrates possess calcium carbonate ( $\text{CaCO}_3$ ) skeletons. Marine plankton, that develop  $\text{CaCO}_3$  skeletons, form the base of the food web and support an abundance of marine life (Sponberg 2007). Most ocean acidification (OA) research so far has been done on coral reefs and plankton, little is known about the short or long term effects of OA on other organisms.

Limpets are gastropod mollusks with a single shell  $\text{CaCO}_3$  and are abundant in the low intertidal around the Puget Sound. As limpet shells dissociate and they secrete a smaller amount of new shell, it becomes more brittle in acidified water. A limpet's  $\text{CaCO}_3$  shell provides protection from desiccation, sun and temperature exposure, predation, and structural support. Limpet fitness should decline as oceans acidify, as they are more easily preyed upon or harmed by environmental conditions such as desiccation. Measuring force from crushing a limpet's shell is a good indicator of shell strength and health because a weakened shell requires less force to crush.

To test whether reduced pH can cause damage to limpet shells when immersed in an acidic treatment, crushing force is measured. Strength of the shell can be an indicator of health and survival potential for the organism. As an acidic environment erodes away and dissolves shells, less force should be required to crush them; the shells should have decreased mass and length the longer they sit in acidified water. I expect to find the weakest shells in the lowest pH treatment after the longest amount of time.

## Methods

The species *Lottia scutum* is the most common limpet found along the Friday Harbor Laboratories (FHL) shoreline and is used in this experiment. Animals with shells 13 mm to 15 mm in length were collected. They are identifiable in the field by short apex height and most organisms are less than 20 mm in length. The shells were collected over a period of two days in the low intertidal in front of FHL. One hundred and fifty limpets were collected and sacrificed for this experiment. After extraction of the limpet from its shell, I measured the initial length, width, apex height and mass of the shell. Equal numbers of shells were submerged in acidified water in each of three glass 3.8 L jars. The water was treated to achieve pH levels of four, six, and a control of unaltered (normal) ocean water, around pH 8. Because the pH is measured on a logarithmic scale, pH levels of four and six demonstrate an extreme change in hydrogen concentration from the control. This extreme pH change allows an experiment to take place in a short time period.

Hydrochloric acid was used to demonstrate the acidification process. This is not representative of actual ocean acidification chemistry, but works to achieve an extreme pH in a short period of time (Summers, A. personal conversation 4/29/11). The pH level was checked with pH indicator strips at least three times a day and was occasionally half a pH point high. The pH was adjusted and brought down to the correct level by adding 0.1 M hydrochloric acid. The shells were suspended in the jar by a mesh screen with a stir bar underneath (figure 4). The water was continuously stirred to evenly distribute the acid and to keep the shells from cupping each other, leaving maximum surface area exposed. The shells sat in the acidified solution for 24, 48, and 72 hours. Forty-five shells

were immersed in each pH and 15 shells were pulled out after each 24-hour interval. As shells were pulled out of the baths, they were blotted with kimwipes™, measured, weighed, and crushed.

The force required to crush a limpet shell was measured after incubation in acidified baths for the targeted lengths of time. The peak force required by the MTS synergie 100 crushing machine to crack the limpet shell was measured with a digital force gauge (in Newtons) (figure 5).

## Results

The shells were visibly more brittle after soaking for 72 hours in a pH of 4. Some shells contained multiple small holes and others were translucent after erosion. These shells required the smallest mean force (15.96 N) to crush when compared to the mean force necessary to crush the control after the same amount of time (29.85 N, figure 1). The peak force needed for crushing limpet shells decreased as pH decreased and as the incubation time increased. Specifically the peak force decreased as the time increased at a pH of 4 (figure 2). A strong correlation exists between mass and length of the shells at hour zero, before treatment ( $R^2 = 0.82$ ,  $P = 0.00005$ ), but is diminished after 72 hours in a pH of 4 ( $R^2 = 0.56$ ,  $P = 0.005$ , figure 3). After this treatment, mass and length decreased from dissolution. The mass decreased proportionally more than the length decreased indicating that dissolution occurred on all surfaces of the shell and not just on edges. For pH 4, most of the change in peak force occurred during the first 48 hours of the experiment ( $P = 0.027$ , two way ANOVA). The change that occurred after 48 hours was statistically insignificant, but after this time the comparison between pH 4 and pH 8

experienced the greatest change in required peak force when compared to any other pH levels ( $P = 0.04$ , two way ANOVA).

## Discussion

The dissolution causes damage and weakness resulting from ocean acidification, as well as mass loss and a reduction of size in every direction: length, width and apex height. Combined with a reduced peak crushing force and increased fragility, limpet fitness and lifespan are threatened. A limpet's shell is important for its survival. Strong calcium carbonate is important for every  $\text{CaCO}_3$  containing organism. Bivalves and gastropods need strong shells for protection from predation and their shell is extremely important for structure. A weakened shell is threatened by crushing predators such as crabs. Also, if too much of an organism's energy is allocated to shell repair, reproduction slows and lowers fitness of the individual and its species.

A weakened shell may also endanger limpets by exposing the organism to desiccation, sun or temperature. Other limpet species would probably not yield the same results because *Lottia scutum*'s apex height is low in comparison; because apex height affects peak force, other species would yield a larger peak force. If *L. scutum*'s abundance decreases, the diversity of other species might increase, shifting species distribution.

Using a rubber pad underneath the limpet shell when crushing for more even force distribution, would make an improvement to this experiment. Larger treatment containers would help hold the pH at a more constant level because of the larger volume to shell ratio. Some slight pH variation occurred daily during the experiment, but only by

half a pH point and was corrected when found during pH testing by adding more hydrochloric acid.

This accelerated acidified treatment is not representative of real life, but demonstrates a dramatic version of what might happen over time if the ocean acidifies. Testing shell strength allows the effects of ocean acidification to be seen on a measurable level, but small changes to organisms could be devastating. For example, if organisms experience recruitment fail, they could die out. Minor fluctuations in the ocean might not seem detrimental for humans but cause major alterations for marine life.

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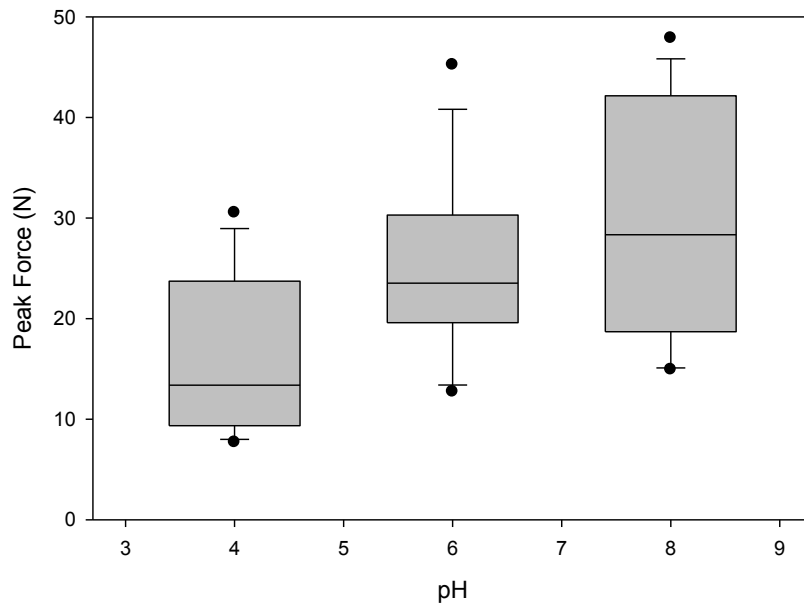


Figure 1. The Peak force (N) required to crush limpet *Lottia scutum's* shells after 72 hours at pH levels 4, 6, and 8

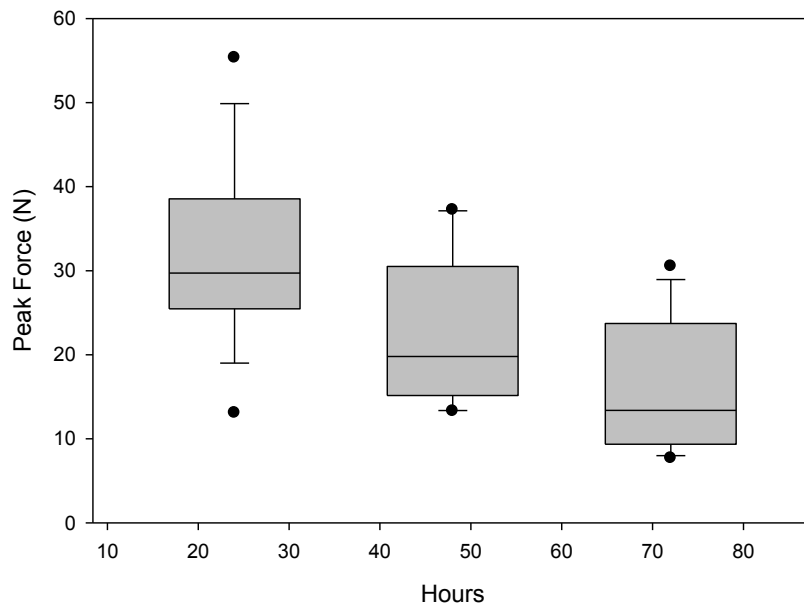


Figure 3. The peak force (N) required to crush limpet *Lottia scutum's* shells at pH 4 after 24, 48, and 72 hours

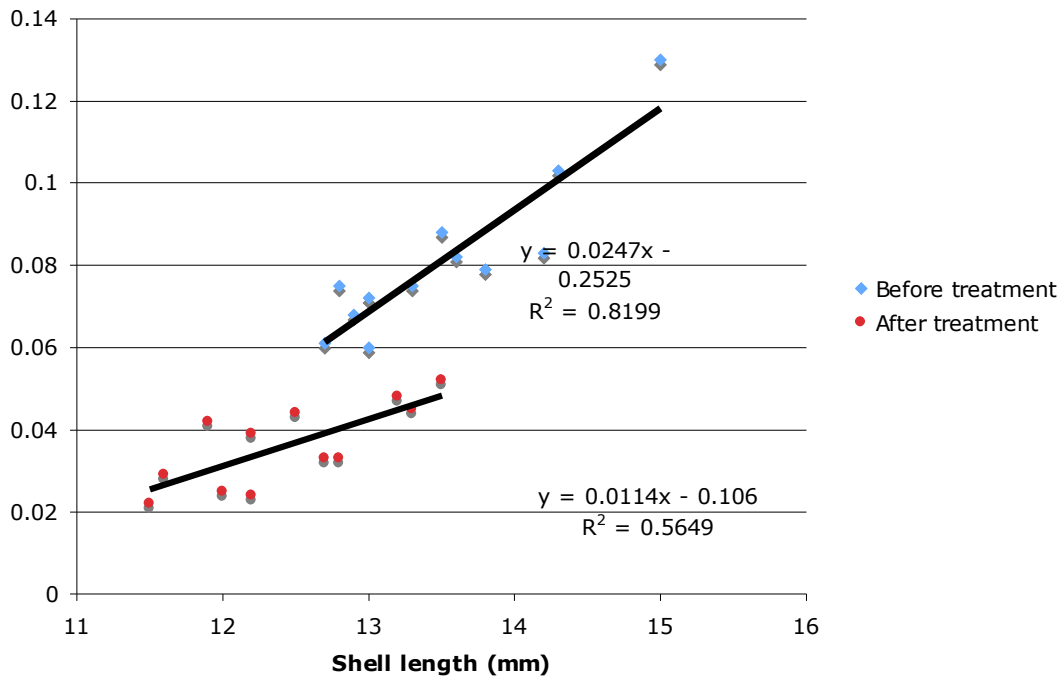


Figure 3. The effect on shell length and mass prior treatment to after treatment in a pH of 4 for 72 hours



Figure 4. Setup of pH baths for shell incubation



Figure 5. MTS Synergie 100 crushing machine with digital force gauge used to measure peak force (N) when breaking limpet shells