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Effect of Ocean Acidification on Crab Shells

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Abstract
This study examines the impact of ocean acidification on Gecarcoidea natalis shell mass by exposing shells to varying pH levels over 30 days. Results show a strong negative correlation between pH and shell mass retention, with the greatest loss at pH 3 . The findings highlight the harmful effects of acidic conditions on calcareous structures, emphasizing the ecological risks of ocean acidification and the need for mitigation strategies.
Aim: To investigate the impact of varying simulated ocean acidification levels on the shell mass of a crab species (*Gecarcoidea natalis)*.

Introduction:
Oceans take up roughly 71% of the Earth's surface and are home to 50-80% of all animal species in the world.[1] Because of its size, the ocean influences climate, sustains ecosystems, and even absorbs 1/4th of the carbon dioxide (CO_2) released by humans.
By being such a massive carbon sink, the ocean can reduce some of the effects of global warming. However, this creates another problem called ocean acidification which involves the rapid decline in pH levels of ocean water. The CO_2 particles react with water molecules in the ocean forming carbonic acid making the water more acidic. The primary cause of ocean acidification is also the primary cause of global warming, the burning of fossil fuels. The combustion of these fuels releases massive amounts of carbon dioxide which is absorbed by the oceans. Other reasons include: Deforestation which reduces the carbon sinks in the world (trees) and increases CO_2 in the atmosphere, and the overuse of synthetic fertilizers which releases nitrous oxide (greenhouse gas) that is absorbed by oceans.
Change in the pH level of ocean water poses a significant threat to marine organisms, many of which depend on calcium carbonate to form their shells and skeletons. Land crabs, for instance, have exoskeletons made of calcium carbonate, which are essential not only for protection but also for maintaining their internal body fluids. Acidic water conditions make it tougher for these creatures to extract carbonate ions, leading to weaker or deformed shells. Given their vulnerability to acidic conditions, land crab shells are an appropriate choice for this experiment.
The typical pH of ocean water is around 8.1. By examining the effects of lower pH levels on land crabs' shells we can extrapolate the impact that varying acidity levels of the ocean water would have on the entire marine ecosystem.[2]

Research Question:
How do different levels of simulated ocean acidification at pH 3,4,5,6,7 affect the percentage change of

[1] "About | UN Ocean Conference | Umoja Wa Mataifa." *United Nations*, www.un.org/sw/node/51825. Accessed 10 Mar. 2024
[2] "About | UN Ocean Conference | Umoja Wa Mataifa." *United Nations*, www.un.org/sw/node/51825. Accessed 10 Mar. 2024

crab shell (*Gecarcoidea natalis*) mass over 30 days?

Hypotheses:
The lower the pH level of the water the higher the percentage change in the crab shell's mass. This is due to the decreased carbonate availability in the more acidic water.[3]

Table of variables:

Independent variables	Dependent variables	Controlled variables
Simulated ocean acidification (pH levels 3,4,5,6,7)	Percentage change in shell mass	Crab species (*Gecarcoidea natalis*)
		Water volume (150mL)
		Time exposed (30 days)

Table 1: Variables

Materials required:
- Crab shells (*Gecarcoidea natalis*) – 10 shells
- Digital weighing scale
- Nylon threads – 25 threads
- White stickers – 30 stickers
- Beakers (200mL) – 5 beakers
- Measuring cylinder – 1 cylinder
- Glass petri dish – 5 dishes
- Glass rod – 1 rod
- Tape
- Aluminum foil
- Hot air oven
- pH meter
- HCl (Hydrochloric acid)
- Safety gloves

Procedure:
1. Wash the crab shells in the sink, then break them into 3-4 smaller pieces. Do this until there are 25 smaller pieces.
2. Weigh a piece of crab shell on the digital weighing scale and note down the initial mass. Then tie a piece of nylon thread to it, put a white sticker on the end of the thread, and label the pH and the shell number (1-5). Repeat for all 25 pieces
3. Using a measuring cylinder, add 150mL water in each of the 5 beakers and label them using the white stickers.
4. Add HCl to a beaker, stir with a glass rod, and check the pH. Continue until the water has a pH of 3. Repeat this to reach pH level 4,5,6,7 in the other 4 beakers respectively

[3] Specktor, Brandon. "Acid in the Pacific Ocean Is Literally Eating Away Crabs' Shells." *LiveScience*, Purch, 29 Jan. 2020, www.livescience.com/ocean-acidification-corrodes-crab-shells.html. Accessed 10 Mar. 2024.

5. Once the pH values of water are achieved, submerge the labeled shells in their respective beakers and tape the threads to the side of the beaker in a way that the label doesn't fall into the water
6. After all shells are in their assigned beakers submerged completely, put aluminum foil over the beakers and leave it for 30 days
7. After 30 days, wear safety gloves, remove the aluminum foil from each beaker, remove all the crab shells
8. Empty the leftover solution in the beakers, in the sink. Take 5 glass Petri dishes and arrange the crab shells based on pH.
9. Let the crab shells dry for 3 hours in the sunlight
10. After 3 hours, cut the threads on the crab shells, and weigh them once again on a digital weighing scale. Note down the final masses.
11. Find the average percentage change for each pH level and draw comparisons between the same

Justification of methods and sampling:
The experiment is done on an organism that is highly dependent on its shell for survival and protection. Hence, one of the impacts of ocean acidification can be extrapolated through this experiment. Focusing on a specific crab species (*Gecarcoidea natalis*) helps reduce the variability of the dependent variable (percentage change in mass) on factors other than the independent variable (pH of water). Also, using 5 crab shell samples per pH level means there is a more accurate representation of the change in mass, ultimately meaning its more reliable. Shells may lose more mass if they are bigger, however, this doesn't always mean the impact on the shell was higher. Therefore, using mass change as a percentage of initial mass gives a better understanding of the impact. Seawater today ranges between 7.7pH - 8.1pH.[4] Therefore, using pH levels 3,4,5,6,7 is effective for measuring the different impacts at various extremes. Hydrochloric acid is a strong acid making it effective for lowering the pH level of water. It also is readily available and cost-effective.

Risks Assessment & Precautions:
- Chemical hazards: Using HCl for adjusting the pH of water comes with its safety hazards, as inhalation of fumes is bad for the respiratory system, and skin contact can cause burns. To mitigate the risk of inhalation the lab will be well-ventilated (windows open). Also, my lab supervisor will help me with this step, as he is experienced in using such hazardous chemicals.
- Safety equipment: While using acidic substances safety gloves were used to avoid the risk of burns.
- General safety: Working alone in the lab as a student is unideal due to my lack of experience. Therefore, I will have a supervisor while conducting the experiment.

Ethical consideration:
- Source of crab shells: No harming of crabs was done to obtain the shells. The crab shells were collected as waste from a commercial shop.
- Disposal of chemicals: the chemicals will be disposed of safely according to school guidelines to avoid any environmental damage.

[4] *EPA*, Environmental Protection Agency, www.epa.gov/ocean-acidification/understanding-science-ocean-and-coastal-acidification. Accessed 10 Mar. 2024.

- Outcome: While some may argue using crab shells is unethical, no matter how it was obtained, the results of the experiment will contribute to our knowledge of the effects of ocean acidification.
- Disposal: The crabs shells were used as compost for the school farm.

Raw Data Table:

	Initial Mass (g)				
pH Level	***Shell 1***	***Shell 2***	***Shell 3***	***Shell 4***	***Shell 5***
3	1.47	1.21	0.69	0.88	1.00
4	0.75	1.35	1.00	0.79	1.00
5	0.93	0.83	1.37	1.04	1.00
6	0.53	0.75	1.59	1.00	0.54
7	1.11	1.09	1.04	1.12	0.92

Table 2: Initial mass of shells

	Final Mass (g)				
pH Level	***Shell 1***	***Shell 2***	***Shell 3***	***Shell 4***	***Shell 5***
3	0.94	0.76	0.40	0.53	0.47
4	0.36	0.61	0.77	0.76	0.64
5	0.87	0.82	1.32	0.98	1.00
6	0.51	0.74	1.57	1.00	0.52
7	0.93	1.10	0.96	0.82	0.86

Table 3: Final mass of shells

Processed data:

Calculate the mean shell mass for each pH level (sum of all shells/5) for initial and final mass
Example: (1.47+1.21+0.69+0.88+1.00)/5 =1.050g
Then find the mean percentage weight change for each pH level using the formula:
[(Final mean mass- Initial mean mass) / Initial mean mass] *100
Example: [(0.620 – 1.050) / 1.050] * 100 = -69.35%
Then find standard deviation using formula:

$$\sigma^2 = \frac{\Sigma(x_i - \mu)^2}{N} = 0.269$$

PH Level	***Mean Initial Mass (g)***	***Standard deviation***
3	1.050	0.269
4	0.978	0.213
5	1.034	0.182
6	0.882	0.393
7	1.056	0.073

Table 4: Mean mass before 30 days

PH Level	*Mean Final Mass (g)*	*Standard deviation*
3	0.620	0.200
4	0.628	0.148
5	0.998	0.174
6	0.868	0.394
7	0.934	0.100

Table 5: Mean mass after 30 days

PH Level	**Percentage change in mass (%)**
3	69.35
4	55.73
5	3.61
6	1.61
7	13.06

Table 6: Percentage change in mean mass of shells

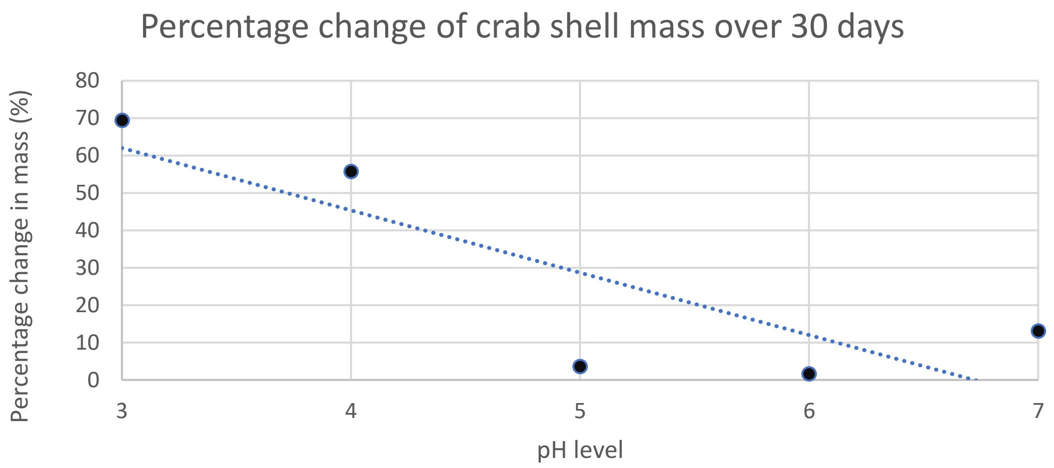

Figure 1: Percentage change in shell mass for each pH level

After plotting the data in a scatter diagram, we see a negative trend between pH levels and percentage change in crab shell mass. As the pH levels increase the percentage change in mass falls. The steepest fall in percentage change is from 4pH → 5pH, from 55.73% to 3.61%. The data also has an outlier where the pH and percentage change have a direct relationship. This is seen from 6pH → 7pH where the percentage change rises from 1.61% to 13.06%.

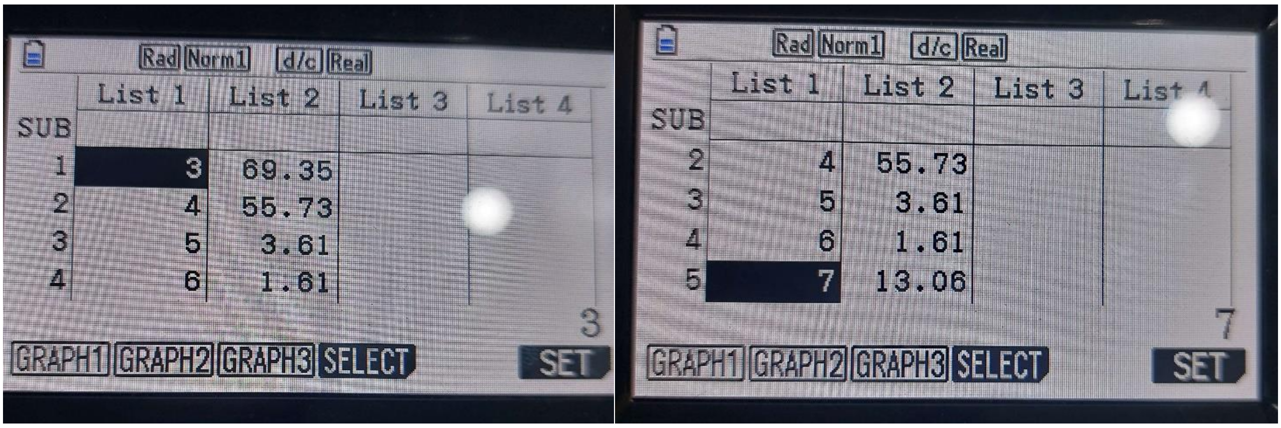

Figure 2: Pearson's correlation calculations

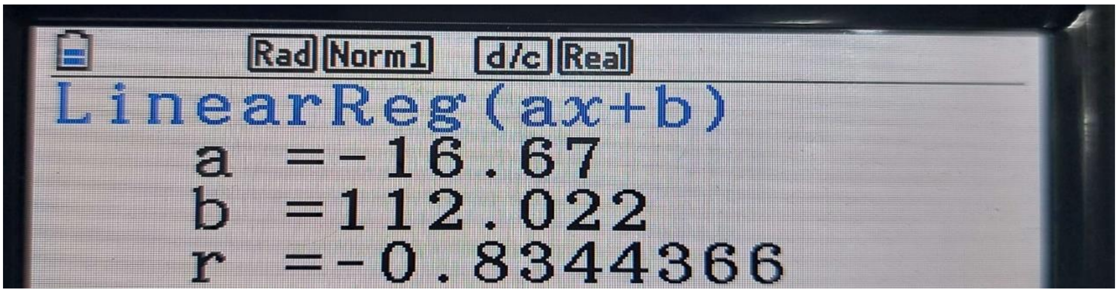

Figure 3: Final Pearson's correlation value

Using the values of percentage change in shell mass and its pH we get the regression of the data. Pearson's correlation coefficient helps us understand the statistic relationship between the dependent variable (change in shell mass) and the independent variable (pH level). The correlation coefficient is -0.834, which implies there is a negative correlation between the variables. Essentially, the mass of crab shells will decrease if the water becomes more acidic.

Conclusion:

My research question explores the correlation in pH levels and change in mass of crab shells. From the experiment, I can conclude that lower pH levels lead to more percentage change in mass for shells. Using Pearson's correlation coefficient (R-value) as a statistical test, we get a value of -0.83, meaning there is a strong negative correlation between pH levels and percentage change in mass. At pH of 3 the percentage change in mass was the highest at 69.35%, whereas at pH of 7 the percentage change in mass was only at 13.06%. This proves my hypothesis that as water becomes more acidic (lower pH level) the mass of the crab shell will decrease. Hence, answering the research question that acidic conditions lead to loss in mass of the shell, loss of color, and degradation of the crab shell.

Discussion:

From the conclusion, we see a negative correlation between pH levels and the percentage change in mass for shells. This further proves that ocean acidification has detrimental effects on shelled organisms. Many shelled creatures, including crabs, rely on carbonate ions in the water to build their shells. In acidic water, there is a decreased carbonate availability, which not only means fewer free carbonate ions for organisms to build their shells with, but also excess hydrogen ions that can dissolve existing carbonate ions weakening the shell.[5] A research paper[6], which studied the impacts of ocean acidification on marine fauna, found that the ability of marine animals to produce calcareous skeletal structures is directly affected by seawater CO_2 chemistry. The experiment that I conducted aligns with this research and the research paper as well, proving that ocean acidification is a serious environmental issue that needs immediate action.

Evaluation:

Strengths of the investigation-

The experiment uses 5 different trials and takes the average; this enhances the reliability of the results obtained. Conducting multiple trials reduces the impact of outliers and random errors, leading to more consistent and dependable results. Having a controlled environment where the only variable was the water's pH level, helps gauge the impact of the water's pH level specifically on the crab shell. This method isolates the independent variable, eliminating the potential effects that could arise in a natural environment, such as temperature fluctuations and salinity changes. Consequently, the findings can be more accurately attributed to changes in pH. The duration of the experiment is just over 1 month. Hence, we can identify the gradual changes that occur including the weakening or erosion of shells, providing a better understanding of the long-term effects of ocean acidification. The experiment is also very simple. By following a straightforward design, the study can be easily repeated with different pH levels or using shells from other marine organisms, making it a versatile model for future research

Weaknesses of the investigation-

Because each crab shell was broken into pieces, the size and weight of each shell sample was different. This inconsistency can lead to unreliable results, as differences in mass and surface area may influence how the shells interact with the acidic water. To increase accuracy, we need to standardize the samples, in other words the weight of each shell sample should be consistent. This would help lower the standard deviation of the results, leading to more precise conclusions. Another limitation is the presence of uncontrollable factors, such as variability among individual crab shells and reactions with the air. These factors could introduce variability that may not be directly related to the pH levels, thus skewing the results. This would explain why the crab shells in the 7pH solution had a larger percentage change in mass than the shells in the 6pH solution. To increase the reliability of the results the experiment could have a larger sample size, perhaps 50 samples, or the duration of the experiment could be increased further, for multiple months or years.

Further scope of research-

The experiment can be repeated with different shelled organisms with higher number of samples. Comparing the results across different species would help identify which organisms are most vulnerable to changes in pH levels. Using a higher number of samples can help reduce the impact of outliers on the conclusion. We can also repeat the experiment at different decimal pH levels to get a better understanding of the relationship between pH levels and percentage change in mass. Experimenting at smaller decimal intervals of pH (e.g., 7.0, 7.5, 8.0) could offer more precise insights into how slight changes in acidity impact shell integrity. This would help detect more subtle trends and patterns in the relationship between pH and shell degradation. The experiment may also be conducted with the independent variable as the

[5] Bennett, Jennifer. "Ocean Acidification." *Smithsonian Ocean*, 11 May 2023, ocean.si.edu/ocean-life/invertebrates/ocean-acidification#:~:text=First%2C%20the%20pH%20of%20seawater,to%20build%20shells%20and%20skeletons. Accessed 15 June 2024.

[6] Fabry, Victoria J., et al. "Impacts of Ocean Acidification on Marine Fauna and Ecosystem Processes." *OUP Academic*, Oxford University Press, 1 Apr. 2008, academic.oup.com/icesjms/article/65/3/414/789605. Accessed 04 Sept. 2024.

number of days, and pH level can be a control variable. This way we can find out how time affects the mass of the shell.

Application:

Ocean alkalinity enhancement is a promising potential solution to ocean acidification. Addressing both the root cause (excess carbon emissions) and its impact, this method involves adding alkaline substances (like as calcium carbonate) to seawater to increase its alkalinity. By doing so, a series of chemical reactions is triggered that reduces the concentration of free carbon dioxide in the water. This creates a deficit, which encourages the ocean to absorb more carbon dioxide from the atmosphere, increasing its carbon storage capacity.

This solution's key strength is its dual benefit. Encouraging the ocean to absorb more carbon dioxide, it helps lower the overall concentration of this greenhouse gas in the atmosphere. This is crucial, as excess carbon dioxide from deforestation, fossil fuel combustion, and industrial activities is a significant driver of climate change. Second, by converting carbon dioxide to carbonate ions, this method directly addresses the issue of ocean acidification. By increasing carbonate availability, it can help maintain the structural integrity of shells and skeletons of marine organisms that depend on these ions.

However, ocean alkalinity enhancement is still in the early stages of research and development. Due to it being a relatively new idea, large-scale implementation hasn't been attempted and potential environmental impacts are still being studied. Trying to manipulate ocean chemistry is extremely risky and can lead to extreme consequences. For example, altering alkalinity could disrupt the marine ecosystem and food webs.

In conclusion, while ocean alkalinity enhancement is a potential solution for mitigating ocean acidification and reducing atmospheric carbon dioxide levels, it remains a new and experimental concept and its long-term impacts on marine ecosystems are not yet fully understood. Therefore, its implementation at this moment is very risky, but in the future with careful evaluation, it could be a sustainable strategy to address ocean acidification.

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