Ocean Acidification, Simulated Statistical Investigation

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Abstract

This study was motivated by the primary investigator's love for the environment. Specifically, an interest in ocean acidification led to the process described in which common brine shrimp were used to simulate the impact of acidification on the world's oceans. Through the use of white, distilled vinegar acting as the "acidification" for the simulated conditions, three populations of brine shrimp were monitored before, during, and after varying levels of acid were added to their individual habitats. Analyses of the data utilizing the Kruskal-Wallis Tests and Chi-Square Test for Association were implemented to compare the effect of acid levels on brine shrimp survival. The results demonstrated statistically significant differences in the survival between the populations of brine shrimp as a function of varying acid concentrations utilizing the Kruskal-Wallis method. Further, the results of the Chi-Square Test for Association demonstrated an association between the acid levels and brine shrimp populations. This investigation is significant because it provides insight into the damage that ocean acidification can cause to oceanic organisms and is demonstrative of the impact humans might be having on our global environment.

Key Words

Ocean, Acidification, Statistics, Correlation, Chi-Square

1. Introduction

This investigation studied the effects of human-induced acidification of the earth's oceans in order to prove a specific point: namely that ocean acidification is harmful to our global environment and that continued increases in carbon dioxide levels as a result of human pollution are unsustainable and detrimental to the world.

Throughout the course of human habituation on earth, humans have seemingly thrived. While the constant advancement in human innovation has led to improved human livelihood, these advancements have also taken a huge toll on the environment. To study these issues, the growing environmental impact of ocean acidification was investigated. Specifically, simulated conditions were created to monitor common brine shrimp survival in controlled environments with varying levels of acidification. The world's oceans absorb approximately one-third of the world's carbon dioxide supply (Fabry, V., *et al*., 2008), and the absorption of this carbon dioxide comes at a cost.

Ocean acidification, or "the other CO_2 problem," is a major problem that has accompanied the increase in human populations and the resulting carbon dioxide levels for centuries, worsening as human populations continue to grow (Doney and Scott C., 2008). As a result, ocean acidification has contributed to the destruction of organisms, habitats, and ecosystems in our world's oceans. Further, ocean acidification has led to numerous mass extinctions (Guinotte *et al.,* 2008) as well as a decrease in calcification, the process by which organisms create calcium carbonate, a critical component of marine animal's shells. For example, ocean acidification was likely responsible in part for the Cretaceous-Paleogene extinction (Anthony, *et al., 2008)*. A decrease in calcification means that Cretaceous organisms are unable or less likely to be able to produce their protective shells. Because ocean acidification is such a prominent problem but receives so little attention, the main study objective was to simulate the effects of increased acid levels on our ocean on marine wildlife.

2. Relationship between shrimp and acidity

Throughout this section, the specific methods and materials used by the researcher will be outlined. From background research, the investigator formed a hypothesis from which they compared their results to. In this section, the methods and procedure will also be explained. The procedure will describe the process followed in this investigation and will explain the general steps of the investigation as they were completed.

2.1 Materials and Procedure

The materials used included one vial of crystalized brine shrimp eggs, three ounces of brine shrimps eggs (one ounce per tank), nine cups of brackish water (three cups per tank), three, four-inch strips of blue masking tape (to create quadrats), and one heating pad (to maintain controlled temperature). In addition, one air pump base, one plastic tube (to distribute air), one packet of inactive yeast (to feed the shrimp), one pair of scissors (for cutting tape), one plastic ruler (for measuring where to put the tape), one fine-mesh net, three labels ("none," "medium," "most"), one ¼ teaspoon of distilled white vinegar, and one tablespoon of distilled white vinegar were all used.

Common brine shrimp were hatched and grown in three different tanks for one month. After they were fully grown, the researcher measured the average population size per quadrat using the quadrat method. Next, various levels of acid (in the form of white vinegar) were added to two of the three tanks.

The first tank was left without the addition of white vinegar to establish a baseline environment for comparison. The second tank was given a medium amount of vinegar (¼ teaspoon), and the third tank was given the most vinegar (1 tablespoon). The amounts of vinegar were chosen for convenience and due to the pH of vinegar in relation to the size of the brine shrimp. The researcher then recounted the brine shrimp and calculated the resulting population sizes (per quadrat). Subsequently, statistical analyses were undertaken as described below.

2.2 General Hypothesis

The hypothesis was that an association and relationship existed between the levels of acid and population sizes of the brine shrimp for individual quadrats in a controlled environment. The expected results were that, when first counted, the population sizes (without vinegar) would be very similar amongst all three tanks (that is, that there would not be a statistically significant difference between them with respect to the measure of interest). It was expected that when vinegar was added, the tanks with a medium and large amount of vinegar, or acidification, would demonstrate proportional decreases in brine shrimp population sizes. It was expected that the tank with the "medium" amount of added vinegar/acid would decrease but that the tank with the most added vinegar/acid would decrease the most where a large amount, if not all, of the brine shrimp would die due to the high level of acidity.

2.3 Data Collection

The researcher utilized several methods to collect data and information. The first step was to hatch the shrimp using an air pump due to the fact that if they did not have access to oxygen, the brine shrimp would have died. However, apart from hatching them, they were very difficult to harm. By using brine shrimp in this experiment, it showed that ocean acidification can affect even the heartiest and most self-sufficient organisms, further proving that ocean acidification is detrimental. This investigation proves that ocean acidification, if not controlled, has the ability to kill off even the most resilient ocean-dwelling organisms.

Approximately one ounce of crystallized, dehydrated brine shrimp eggs were added to each of the three tanks. Hatching occurred within 24 hours. After securing the aeration system, the brine shrimp grew for about four weeks and were fed the same amount of yeast two times a week for all four weeks. Once they reached the point where they were matured, quadrats were taped on and were evenly spaced. The number of brine shrimp for a given quadrat was then counted thirty-one times. Only one quadrat out of four was counted for each tank. They were all in the same position, and **only shrimp in this one quadrat were accounted for in the thirty-one samples**. That is, the quadrat that was accounted for was kept constant.

After recording this baseline data, the vinegar was added as specified (none, ¼ teaspoon, and one tablespoon). White, distilled vinegar was chosen due to its general availability and acetic acid content, which has a pH of about 2.4 making it acidic while still safe for the brine shrimp. It is also important to note that several factors were kept constant so as not to disrupt the data. Specifically, the temperature of the water was kept stable, the amount of food for each of the tanks was kept the same, the water level for the tanks was the same (to begin with), and the availability of oxygen was the same for all three tanks. The next step was to recount how many brine shrimp were in each quadrat thirty-one times after the vinegar had been added. This data was then used to estimate the population sizes.

Next, statistical tests were run comparing the medians of the three shrimp tanks **per quadrat** without the addition of acid (baseline) and after the addition of acid (test articles). The purpose of these tests was to assess for statistically significant differences between the brine shrimp populations per quadrat and will be elaborated in a later portion of this paper.

3. Data and Statistical Analyses

The Kruskal-Wallis Statistical Test and the Chi-Square Test for Association were used to analyze the data collected in this experiment. Tables and figures were also used to showcase the organized data. The section will be broken down into Data Presentation (the figures and tables), the First Kruskal-Wallis test, the Second Kruskal-Wallis test, and the Chi-Square test for Association. Each section for which a test was run will include information on the analyses of the data and subsequent results. The Data Presentation section is useful in visualizing data as well as shows the actual data collected.

3.1 Data Presentation

Figure 1. Brine Shrimp Populations (After Vinegar)

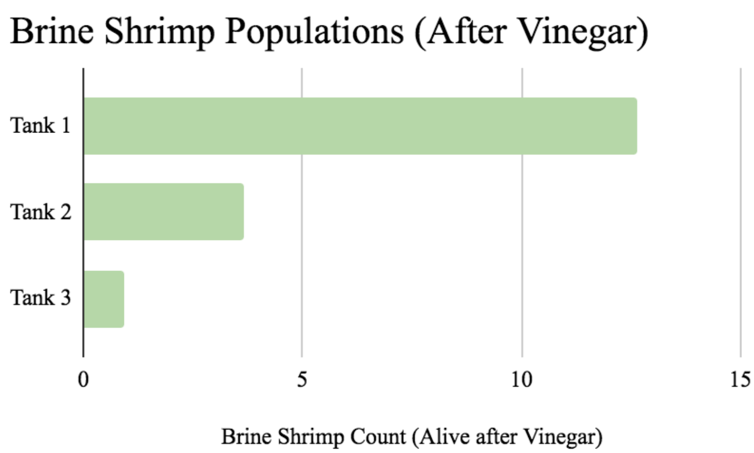

Table 1. Population Size (Per Quadrat) In Contingency Table (For Association Assessment)

Population Size (Per Quadrat) In Contingency Table (For Association Assessment)

ACIDITY LEVEL

	MANY	MEDIUM	FEW/NONE
NONE	6	25	0
MEDIUM	0	25	6
MOST	0	3	28

For Table 1, quadrat measurements with between 0 and 2 brine shrimp fell into the "few/none" category. Quadrats with between 3 and 16 brine shrimp fell into the "medium" category. Finally, quadrats with brine shrimp numbers between 17 and 31 fell into the "many" category. The raw data for the tanks after vinegar was sorted through and the data for each of the three tanks was then categorized into three labels (many, medium, and none/few).

There was a general independent variable and dependent variable. The independent variable overall was acidity level. Because acidity level was measured on a scale of none, medium, and most, this was a form of ordinal scale data. Ordinal scale data can be defined as the type of scale data used when the variable is measured in discrete categories. Fit to this experiment, the levels of acid were measured on a scale that implied one tank held no vinegar and another tank held the most vinegar. Therefore, the levels of vinegar were ranked on a scale with defined rank order. Secondly, the dependent variable was the population size of shrimp for an individual quadrat. The quadrats that are classified as having "many brine shrimp" imply that there was a greater amount of brine shrimp in said quadrat than there was in the quadrats classified as "none/few" because one would assume the none/few category to hold very little, if any, brine shrimp.

3.2 Kruskal-Wallis Assessment for Means of the Medians Part 1

The Kruskal-Wallis test is a statistical test used to compare the medians of three or more samples. The Kruskal-Wallis test was used here because, unlike its parametric analog, the ANOVA test, the nonparametric Kruskal-Wallis test can handle ordinal scale data. This is relevant because, as stated, the data collected was on the ordinal scale. More specifically, the Kruskal-Wallis has the ability to detect if there is a statistically significant difference between groups of data.

To begin, the Kruskal-Wallis test was used to determine whether there was a statistically significant difference between the means of the medians of populations of shrimp per quadrat of all three tanks **before** any vinegar was added. The purpose of running this test was to establish a baseline that would later show that when vinegar was added in medium and great amounts, proportional changes in numbers would be observed. The primary reason why the brine shrimp populations decreased was assumed to be the addition of vinegar increasing the acidity of the test environments. The analysis shows that the drop in populations per quadrat was not because the tank with the most vinegar had less shrimp to begin with, but instead the drop was a direct result of adding vinegar/acidifying the environment. As a result of running this test using VassarStats, an H score of 4.88 was obtained. This 4.88 was then compared to the appropriate critical value listed in Appendix 3 of the Practical Statistics for Field Biology book. The appropriate critical value for a degrees of freedom (sample size minus one) of 2 is approximately 5.99, which is greater than 4.88. Because 4.88 does not exceed the critical value of 5.99, one fails to reject the null hypothesis that the result was not statistically significant. Thus there was no statistically significant difference between the mean population sizes of the shrimp per quadrat in each of the three tanks prior to adding any vinegar. This is significant because it shows that the later decrease in population sizes was due to the addition of vinegar, not because the tanks had different levels of shrimp to begin with.

3.3 Kruskal-Wallis Assessment for Medians Part 2

The second Kruskal-Wallis test was used to compare the medians of the three tanks **after** their specific levels of acid were added. This test was used to prove that there was a statistically significant difference between the populations of brine shrimp per quadrat with the addition of vinegar. VassarStats was once again used to run the Kruskal-Wallis test. A very high H score of approximately 73.89 was obtained. After comparing this H score with the same critical value as prior (5.99) because both tests had the same degrees of freedom (2), and, recognizing that 73.89 greatly exceeds the critical value of 5.99, the null hypothesis was rejected and instead the alternative hypothesis was accepted. The alternative hypothesis was that there was a statistically significant difference between the means of the medians of the average population of brine shrimp per quadrat in these tanks. This is important because it proves that the acid added directly affected the brine shrimp in that by creating an acidic environment, the organisms living in it likely struggle to survive.

3.4 Chi-Square Test for Association

The second analysis utilized the Chi-Square Test for Association. The Chi-Square Test for Association is a statistical method used to figure out whether two variables are associated. The Chi-Square Test for Association uses frequency data. Since this study's data was collected as individual samples, the raw data had to be converted into frequency data. After the data was correctly converted, the Chi-Square Association test was run on VassarStats.

As expected, the chi-square value turned out to be very high, supporting the hypothesis that there was a direct association between levels of vinegar and populations of brine shrimp per quadrat. It came out to be 68.62. To assess this score, the critical value listed in Appendix 3 under Degrees of Freedom of 4 was assessed. This showed that the correct critical value for the 0.05 p-value was 9.49. Because 68.62 so greatly exceeded 9.49, the null hypothesis was rejected to show that there was no association between the two variables and the alternative hypothesis (that there was a direct association between levels of vinegar and populations of brine shrimp per quadrat) was accepted. This proved the hypothesis that there was an association between these two variables. This is important because it shows that the addition of vinegar to the brine shrimp tanks directly affected the survival rate of the shrimp, suggesting that ocean acidification decreases the survival rate of organisms.

4. Conclusions

From these statistical results, one is able to understand that a Kruskal Wallis test run between the medians of the populations of brine shrimp per quadrat **before** the addition of vinegar does not produce a statistically significant result. Thus, it is most likely that the drop in population sizes was not due to an outside factor but due solely to the addition of white vinegar. The second Kruskal-Wallis test run between the populations of brine shrimp (in medians) after the addition of vinegar demonstrated a statistically significant difference between the medians of the populations of brine shrimp once vinegar was added. Finally, the use of a Chi-Square Test for Association shows that there was, in fact, an association between the levels of acid in the three tanks and the amount of brine shrimp that remained living. Overall, the data did support the original hypothesis that there was an association and relationship between the levels of acidity and the populations of brine shrimp for each quadrat.

The conclusions that can be drawn from the statistical analyses done are numerous. To begin, one is first able to conclude that there was an association between the levels of vinegar in the brine shrimp tanks and the populations of the brine shrimp (per quadrat). This is important because it supports the conclusion that, on a larger scale, ocean acidification directly affects organisms. If this study were to be replicated on a larger and more complex scale, it is likely that it would produce similar results. One can hope that if more people were made aware of such findings that they would take more steps toward stopping ocean acidification. Further, this experiment also allows one to draw the conclusion that vinegar is in fact acidic and that even a low acidic pH such as 2.4 can be detrimental to a population. That is, one is able to conclude that even a low acidic pH can make a large difference.

5. Future Research

Possible further investigations that could take place based off of this research if a scientist were trying to raise awareness about the effects of ocean acidification. That is, based on this research, people could begin to realize the harm in ocean acidification and begin to attempt to heal it as opposed to continuing to produce thousands of tons of carbon dioxide each year. Though this is far easier said than done, this investigation would be a good springboard for a much larger scale investigation completed at a much higher level to prove that ocean acidification is a global issue.

Through undertaking additional similar experiments on a larger as well as far more complex scale, scientists may be able to discover possible solutions to ocean acidification as well as gain a better understanding as to how such low levels of acid can adversely impact our environment. Since current research predicts that ocean acidification will cause harm in the future (Pörtner H, 2008), it is imperative that further investigations for a solution begins now. As humanity continues to advance technologically and produce more waste as a population, it is vital to understand how human waste has and is affecting the environment and to ponder potential solutions. Though ocean acidification is only one issue arising from our species and its growth, it is still of critical importance. Perhaps through a better understanding of this and other environmental changes that we are inducing globally may have a chance to stop damaging our oceans and our planet before it becomes too late.

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Biography

Emersen M. Panigrahi is a student at Stanford Online High School. Besides being a student interested in marine life and the effect of humans on the environment, she also finds herself interested in biological sciences and genetic engineering. Emersen is also a competitive fencer as well as a dedicated, aspiring photographer. She has co-written a speckle interferometry paper and is currently working on a medical paper. In addition, Emersen is currently attempting to enter the world of business as she prepares to start her first business as she and her siblings attempt to launch their first charitable website. Emersen is thankful for the opportunity to publish this paper!