I. Summary:

Human, during their rapid development process, has left unneglectable traits to their surroundings. Reportedly, since the Industrial Revolution, the ocean's pH level has lowered by 0.1 pH units, which translates to a 30% increase in acidity, posing a constant threat to the diverse sealife. In fact, due to ocean acidification, shellfish, whose calcium shells are highly vulnerable to the acidic ocean water, has been dying at an alarming rate of 60-80 percent in hatcheries (Dolan, 2013). Thus, to explore whether shellfish, in particular Crassostrea gigas, the common oysters in Pacific Ocean, could cope with the increasingly acidic environment, I propose the following experiment, where we look at Crassostrea gigas' shell evolution over time.

II. Hypothesis:

In response to ocean acidification, Crassostrea gigas evolve by increasing their calcium production, resulting in thicker shells.

III. Type of research design:

I propose a standard experiment in which sampling is randomized and blinding is applied on the trialists' side as the preferred approach to our hypothesis for the following reasons:

1. The experiment is comparative. By establishing a control group and an intervention group, we could be ensured that a definite causal conclusion could be drawn from the experiment. Thus, at the end of the experiment, we could effectively conclude the effect our treatment (or ocean acidification) has upon Crassostrea gigas. The experiment would then be much more conclusive than observational studies, in which there is no control group to compare and potential relationships are not readily visible due to nature being a complex system.

2. Randomization of subjects and concealment of their allocation mitigates biases and confounding variables. The result would, hence, be more objective and likely replicable.
3. This is somewhat closer to an RCT than a standard experiment. However, as RCT is normally used human and there is a placebo controlled group, calling the experiment RCT might not be applicable.

IV. Treatment: Manipulating acidification level in ocean water.

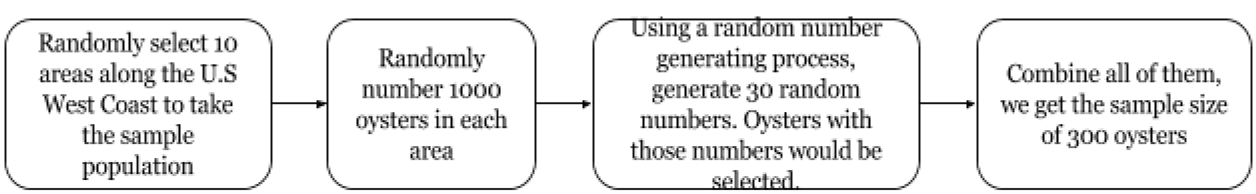
V. Variables:

- Independent variable: acidification level in water.
- Dependent variable: Crassostrea gigas' shell thickness.
- Potential Confounding variables: The appearance of predators, carbonate concentration in seawater.[1]

VI. Narrative description:

1. Collecting sample from the population:

We decided to carry out the simple random sampling to reduce as much bias in choosing the oysters as possible:



While this is a time-consuming and costly process, it ensures that our sample size is representative and bias-free. An alternative to the above sampling method would be to divide the

[1] #experimentaldesign: Identified involved variables and proposed methods to control and manipulate them so that extraneous variables are avoided.

sheltered water areas around the West Coast into grids, each with a number. A computer would then generate a random of set of numbers, which are areas we would take samples from[2].

2. Allocation of test groups and control group:

The allocation of samples to test group and control group is completely randomized and trialists would not be informed of which group they are working on.

3. Pre-test:

Carry out a comprehensive test to measure the shell thickness of each crassostrea gigas and calculate the average shell thickness of each group.

4. Administer independent variables for each of the test group:

Test group #1: Using an ion exchange resin, we manipulate the acid level of this group, we set and keep the acidification level of the water to pH = 7.7

Test group #2: With the same method, we set and keep the acidification level of the water to pH = 8.1 (the current pH level of the ocean)

5. Apply the same environment properties (temperature, oxygen concentration, pressure, light level, etc.), feeding methods and all other potential affected variable for both the test group and control group for 2 years.

6. Post-test:

Carry out the same comprehensive as we did in the pre-test to gather the data for final analysis but for the original samples' offspring.

VII. How bias and confounding variables could be mitigated:

[2] #sampling: proposed appropriate sampling methods that might be used for the experiment.

A standard experiment would minimize confounding variables and eliminates confirmation bias, memory bias, emotional bias and heuristic bias. An experiment, unlike observational studies, makes sure that all potential variables are considered so that they do not have any significant effect to the outcome. As we conduct this research in lab condition, we have even more chance to control variables so that subjects' conditions are identical except for the water's acidification level. This ensures that we arrive at a causal conclusion in the end. An observational study, for example, would completely lag behind in this aspect, as it would not be able to reveal a causation due to the fact that we are extracting information from a complex system (we might never know if crassostrea gigas develop thicker shell to fare off their predators instead of adapting to the increasingly acidic water).

Biases are minimized too. Randomized sampling, for example, helps reduce heuristic bias, or availability bias in particular[3]. As crassostrea gigas might have adapted to each area's living condition, if we select subjects from one area only, we might end up with a biased sample with all similar subjects. This unrepresentative sample size would later on render the experiment uneffective and irreplicable. A good randomized sampling process would, on the other hand, provide trialists with a diverse population of crassostrea gigas in different shapes and sizes, thus strengthening the conclusion[4]. Even though it may be impossible to apply double-blind to this experiment, the fact that trialists do not know which group their subjects are in would help mitigate confirmation bias.[5] This, accompanied with the fact that I aim at a quantifiable,

[3] #heuristicbias: Analyzed ways in which availability bias could potentially skew result and proved how the experimental design could avoid such situations.

[4] #replication: Analyzed the possibility that the research could be replicated in the future by considering how representative and externally valid it could be.

[5] #confirmationbias: identified possible scenarios where confirmation bias may skews the research process and proposed ways in which the experiment could minimize this bias.

recordable and comparative, makes sure that researchers do not come to a conclusion unconsciously as they are convinced of the result from the beginning. Designed to be as objective as possible, the experiment aims at a definite conclusion instead of a subjective one that is influenced by researchers' prejudice.

Another advantage of this type of experiment, in which records are taken, samples are representative and the result is quantifiable, is that memory bias and emotional bias are avoided. A researcher, like any other human beings, might be more interested in outstanding subjects in their experiment. As we tend to remember striking differences instead of the norm, one crassostrea gigas with higher calcium production rate might be more noticeable for researchers as a result. However, as we focus on how one entire species adapts to environmental changes, such a concentration on a singular subject would be skewed and not conclusive. This experiment, with its holistic view and systematic approach, rules out the fallibility of memory in recording and analyzing procedures[6]. Regarding emotional bias, this experiment minimizes the probability in which researchers' grudge toward climate change and pollution makes them hastily jump to a conclusion. Measuring the shells' change over a long period of time, with trialists not knowing which group they are following, the research encourages researchers to carefully consider possible nuances that might be observed and fosters a non-biased outcome[7].

VIII. Expected result:

After conducting the experiment, we expect that the acidic ocean water would, at first, make subjects' shells thinner as it dissolves shells' carbonate structure. However, over time, the

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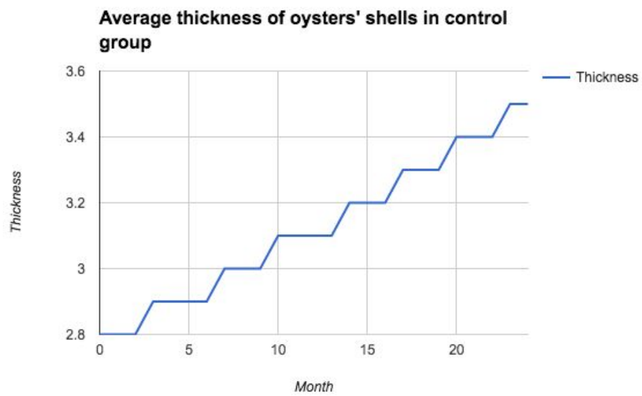
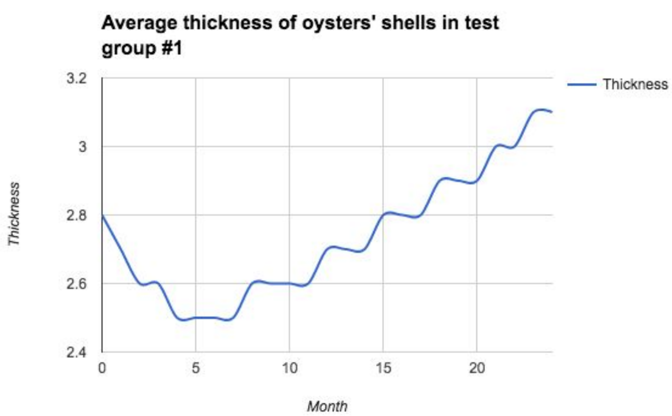
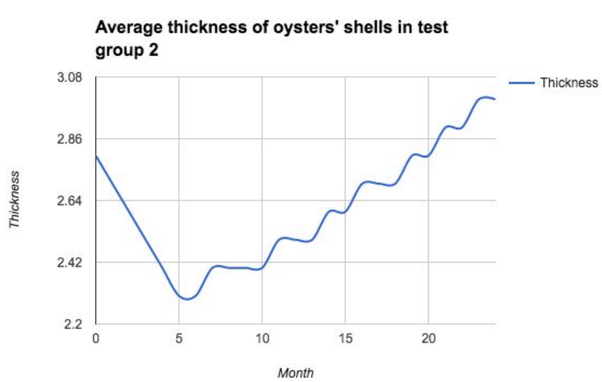
[6] #memorybias: looked at the mechanism of memory bias and analyzed how it might be avoided.

[7] #emotionalbias: Emotional bias could potentially influence our decision making process, altering our risk perception in particular. I analyzed how emotional bias could be involved in this experiment,

crassostrea gigas would adapt to the environment and build thicker shells. Expected results are plotted in the following graphs. I use an arbitrary thickness unit to better visualize the outcome.







IX. Reference:

Dolan, M. (2013, February 18). The Pickled Ocean Is Eating Away at Oysters, Clams, and Sea Butterflies. Retrieved February 28, 2017, from http://www.slate.com/articles/health_and_science/animal_forecast/2013/02/ocean_acidification_and_oysters_shellfish_are_already_suffering_1.html