I. Introduction:

Barnacles are usually small, sessile crustaceans that are frequently seen in the higher inter-tidal zone of rocky tide pools and along docks/pilings which are periodically and daily covered with sea water. Though often overlooked below the feet of visitors to the marine environment, these animals are routinely making a living by sweeping sea water for small particles and organisms from the plankton of the ocean. Barnacles are suspension feeders using a hand like apparatus called a "cirri" that can be observed with a simple magnifying lens or dissecting scope. Enclosing the body of this animal is a calcium carbonate covering/shell that is important for protection from the elements and desiccation prevention.

Ocean acidification is a phenomenon that has been studied intensively for approximately the last decade by geophysicists, chemists and oceanographers. Scientists have been monitoring the pH of the ocean using very precise instrumentation to see if there is any effect of increasing levels of carbon dioxide CO$_2$, from both natural and human derived sources such as the burning of fossil fuels. The ocean is typically slightly basic, having a pH of approximately 8.2. When CO$_2$ dissolves in water it has a slight acidifying effect, turning some water particles into carbonic acid H$_2$CO$_3$. Scientists from the University of Southern Florida's College of Marine Science using very precise instrumentation compared pH levels over a span of 15 years, a very slight, though statistically significant decrease in ocean pH was noted. Previously fresh water acidification, which has been noted in many North American lakes, rivers and streams has been the cause of changes to fresh water food webs, especially with respect to animals that have calcium carbonate shells such as clams and snails. Very little study has been carried out to date about the potential effect of acidification of marine animals that have calcium carbonate shells or tests.
II. Problem:
This investigation will explore the potential effects of acidification on feeding rates seen in the ubiquitous barnacle to allow students the opportunity to determine if slight acidification of marine water has an effect on one model organism that is dependent upon a calcium carbonate shell.

III. Hypothesis:
Complete the following statement using a cause and effect approach that can be tested using the methods of science.

"If barnacles are exposed to different levels of pH in ocean water then –

__________________________________________________________________________ ."

Hint: What do you expect to happen?

IV. Materials:
Live barnacles attached to rocks or other suitable substrate.  Hand counters
Watch glasses  Timer
pH meter  Concentrated plankton
Sea water  4 Transfer pipets
Acid bottle  Graduated cylinder
Dissecting scope  Glass stirring rod
Hand counters  Marking tape & pen

V. Methods
Please read through all of the following procedures BEFORE you begin the study.

i. Preparation of chambers

1) Your team should obtain 4 glass watch bowls.
2) Measure equal amounts of sea water into each of the bowls – 100 ml recommended if the bowls are large enough. The water should be deep enough to completely submerge 3 barnacles on their rock.
3) One bowl will not be treated or have the pH changed, this is the control.
4) Record the pH of the non-treatment bowl in the table on pg 3.
5) Next, each of the bowls will be modified by adding 5 drops of acid at a time, stirring after every addition of 5 drops with a glass stirring rod.
6) Continue to add acid until you are approximately at the following pH levels for each of the bowls respectively. Test the pH after each addition of 5 drops of acid.

6.5 >>>>> Most acidic
7.0 >>>>> Moderate acid
7.5 >>>>> Least acidic treatment
7) Record the pH of each treatment in the table provided.
8) Label each of the bowls with a pen & lab tape regarding their pH status and the control's pH.
9) Place each bowl on a dissecting scope in a position that is easy for a student to monitor and count barnacle activity on (ideally while in a seated position).
10) Place three barnacles into each of the treatment bowls.
11) Observe the one barnacle and sketch its appearance and power level in the results section to become familiar with the morphology of this animal.
12) Turn off the light on the dissecting scope and let the barnacles adjust to their new situation for at least 3-5 minutes.

ii. Obtaining data
> Read all of the following instructions before you begin, begin only when your instructor starts the clock.

> Each student will monitor one pH treatment only, but 3 barnacles within each, choose animals that are easy to see without moving them during the experiment.

> Results per the team will be compiled after data is collected into one graph.

1) Give your treatment one ml of suspended phytoplankton, ideally all team members should give their 1 ml of food to the barnacles simultaneously.

2) Each person will monitor one barnacle (#1) in their treatment for 30 seconds at a time, counting the number of sweeps of the cirri, keep track with the clicker counter. This will allow an easy multiplication of x 2 to get an idea of the rate per minute. Your instructor will keep track of the time so that you don't have to monitor the clock as well as count.

3) There will be a 30 second wait period, where you will record your value in the table on pg 4 and also reset your clicker. During this rest period, shut off the light on the scope so that it does not heat up the water and thus enter another variable into the experiment.

4) Repeat the count for a second, different barnacle (#2) as in the step outlined above in step #2.

5) Give a 30 second rest, shut off the light and record data for barnacle #2.

6) Repeat the count for a third, different barnacle (#3) as in the step outlined above in step #2.

7) Give a 30 second rest, shut off the light and record data for barnacle #3.
8) Carry out this procedure in rotation for each barnacle over several trials, ideally at least 5-10 rotations to obtain sufficient data. Record the feeding rate at each observation. Do not change the sequence. Observe #1, then #2, then #3

9) Graph your results, discuss amongst your team members the best way to represent your data for 3 different barnacles in 4 different environments over time, there is more than one way to adequately represent your data.

**VI. Results**

Barnacle Sketch  
Magnification level _____X  

Feeding Rates of Barnacles  
Data Table  
pH value of this treatment: _______  
Evaluator's name: ______________  

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VII. Analysis

Discuss the following questions as a group, nominate one person to report the results of your collaborative reasoning.

1) Evaluate your hypothesis, was it supported or rejected? Explain using data acquired during this experiment.

2) Did the range of pH values that your group chose or were assigned demonstrate any difference in the behavior of the barnacles?

3) In this experiment you were required to monitor three different barnacles, why not just one i.e. what is the benefit of evaluating more than one animal's behavior?

4) Due to in-class time restrictions you monitored three different animals using four different conditions, to evaluate the potential change in one variable. Identify the different components of the scientific method below used in this experiment.

   > Independent variable
   > Dependent variable
   > Experimental manipulation/variable

5) What are some of the potential problems or errors that may have entered into this experiment that may have been inadvertently caused by human mistakes?

6) What are some of the potential problems or errors that may have occurred due to experimental errors i.e. problems with the procedure or factors outside of the control of the students conducting the experiments?

7) If this experiment were to be repeated with other groups of students in the future, what is one piece of advise that you would give to such a group to improve the experiment?
Turn into your instructor:

> The analysis questions (1 per group), with everyone's names on this sheet.
> Your collective graph of the results.
> The results page of your individual results and sketch (each person submits this page).

**Staple all of the above together and turn in your report as a group.**

Possible Extensions

*If the instructor assigns as an additional assignment, or for your own learning purposes.*

1) Research the topic of ocean acidification from a magazine, journal article or credible i.e. scientific website such as NOAA and provide a one page summary of the article and the citation to that article.

2) Investigate the life history characteristics of barnacles i.e. from zygote > larvae > juvenile > adult. Which stage of the life cycle might be most vulnerable to chemical changes in their environment? Where do barnacles reside? Do barnacles exhibit any food preferences? Are there different species of barnacles in the Pacific North West? etc.

3) Investigate the internal anatomy of barnacles and make a sketch of what you find. How are barnacles similar to other marine arthropods such as crabs, lobster etc and how are they different? Compare and contrast the barnacle's anatomy with a fully mobile crustacean.

4) What other marine animals on the Oregon coast are likely vulnerable to ocean acidification? Investigate if there are any publications on this topic specific to Oregon or the PNW and make a list of such animals. Are any of these animals of commercial use to humans and if so, speculate what measures may have to be taken to protect these resources.

5) This investigation attempted to monitor the effects of only one variable, differing pH values. Barnacles are also exposed to a variety of other variables in their environment including changing temperatures, disturbance, salinity changes, pollution exposure etc. Create an experimental design by outlining how you would assess the effects of a different variable than pH fluctuations. Are there are other monitored effects that you could observe other than rate of cirri sweeps that might give an indication of this animal's reaction to such a variable?