NOAA's *aquarius* America's Innerspace Station

Design Your Own

Focus
Experimental design in coral reef research

Grade Level
9-12 (Life Science/Earth Science)

Focus Questions
Why are experimental designs a critical component of scientific research?

What steps are involved in designing a scientific investigation?

Learning Objectives
Students will be able to explain at least four reasons for conducting a scientific investigation.

Students will be able to formulate a testable hypothesis and explain the logical connections between the design of an experiment and the scientific concepts that are the basis of a hypothesis.

Materials
- Copies of "Global Climate Change & Coral Recruitment: The Interactive Effects of Temperature and Ontogeny on the Biology of *Porites astreoides* Larvae. Mission Summary and Background," one for each student or student group

Audio/Visual Materials
- (Optional) Chalkboard, marker board, or overhead projector for use during group discussions

Teaching Time
Two 45-minute class periods, plus time for student research

Seating Arrangement
Groups of two to four students

Maximum Number of Students
30

Key Words
Experimental design
Null hypothesis

Background Information
Aquarius is an undersea laboratory owned by the National Oceanic and Atmospheric Administration (NOAA). Its purpose is to support research on oceans and coastal resources by allowing scientists to live and work on the seafloor for extended periods of time. *Aquarius* is presently deployed three and a half miles offshore in the Florida Keys National Marine Sanctuary. It operates 62 feet beneath the surface at Conch Reef. Missions typically last 10 days and aquanaut candidates undergo five days of specialized training before each mission starts. Visit http://www.uncw.edu/aquarius/ for more information, including a virtual tour of the *Aquarius* laboratory.

Aquarius missions are focused on understanding our changing ocean and the condition of coral reefs. Coral reefs are threatened locally, regionally, and globally by increasing amounts of pollution, over-harvesting of fisheries, disease, and climate change.

Thermal stress appears to cause some of the most severe damage to coral reefs. Most shallow-water reef-building corals live in tropical latitudes (less than 30° north or south of the equator) where the normal water temperature is near the upper limit of the corals' thermal tolerance. When water temperatures rise above this limit, the result is thermal stress, which causes many corals to expel the symbiotic algae (zooxanthellae) that live in the corals' tissues. Because zooxanthellae provide a significant portion of the corals' food and are involved with growth processes, expelling these symbionts can have serious impacts on the corals' health. In some cases, corals are able to survive short periods of thermal stress and eventually recover. When the level of environmental stress is high and sustained, however, the corals may die. Zooxanthellae are responsible for most of the corals' color, so corals that have expelled their algal symbionts appear to be bleached. In general, coral "bleaching" events often occur in areas where the sea surface temperature rises 1°C or more above the normal maximum temperature.

Despite widespread reports of coral "bleaching" events and increasing public recognition of the problem, very little is known about how temperature stress affects coral reproduction and coral larvae that are critical to reef maintenance and recovery. In June, 2002, a group of scientists led by Peter Edmunds of California State University used *Aquarius* as a base for research directed toward this question. Being able to work from *Aquarius* was critical to this research, since scientists wanted to collect larvae from many coral colonies during both day and night. The scientists planned to investigate three questions:
1. Do adult and larval corals display physiological differences that might make the larvae more (or less) sensitive to changing temperatures?
2. Does larval age and history affect their response to environmental challenges?
3. Do the larvae and adults contain the same types of symbiotic algae?

In this activity, students will formulate a hypothesis for the first of these questions, and will outline a procedure that could be used to test this hypothesis.

Learning Procedure

[Note: This lesson is based on an activity developed by Lisa Wall.]

1. Direct students to prepare a one- to two-page summary of background information on the *Aquarius* habitat, coral reefs, and ways in which these reefs are threatened. Information on the *Aquarius* web site (http://www.uncw.edu/aquarius) and coral reef tutorials at http://www.nos.noaa.gov/education/welcome.html provide "one-stop shopping" for this assignment.

2. Lead a group discussion of students' summaries. Be sure that students understand:
   - The importance of coral reefs;
   - The relationship between coral animals and their symbiotic algae (zooxanthellae)
   - The effects of thermal stress on reef-building corals; and
   - The advantages of the *Aquarius* habitat for certain types of scientific research.

Have students brainstorm and discuss typical reasons for undertaking scientific investigations. These reasons should include:
- Exploring new phenomena;
- Confirming previous results (a very important part of the scientific process; results must be repeatable by other scientists to be considered valid);
- Testing the predictive value of a theory; and

- Comparing different theories concerned with the same natural phenomena.

3. Review the classic steps in conducting a scientific investigation:

a. Posing Questions – Appropriate questions are ones that can be answered by gathering evidence.

b. Developing Hypotheses – Hypotheses are predictions about the outcome of an experiment, and must be testable. Scientists often phrase their predictions as "null hypotheses, "which state that there will be no difference between experimental and control groups when the experiment is performed or observations are made.

c. Designing Experiments and Observations – This involves preparing a step-by-step plan that describes specific observations, manipulations, or measurements that will be made to test the hypotheses. The experimental design should identify relevant variables and how these will be controlled. In an ideal investigation, all variables are kept the same but one, which is called the manipulated variable. In practice, it is often impossible to keep some variables the same. If an investigation is studying the difference between two species of snails, each snail that is studied will be different in some ways from others, even if they are from the same species. Similarly, time, weather, lunar cycles, and many other factors can be difficult to control if observations are made at different times or on different days. Investigators need to keep these kinds of variables in mind when they begin to interpret their results.

Another important aspect of experimental design is developing operational definitions that specify the meaning of certain terms or how certain variables are to be measured. For example, an investigator who wants to examine the effects of temperature on an organism has to decide what observations or measurements are to be made that would indicate an "effect." Increased respiration, changes in food consumption, and death are among the many observations that might be used to indicate an "effect."

It is critical for the experimental design step to be extremely detailed, so that others can replicate the investigation to determine whether the results can be confirmed. Without this detail, the investigation loses a great deal of value.

d. Conducting Experiments and Making Observations – Once experiments begin, it may become clear that the experimental design needs to be modified. Again, it is critical to document any such changes so that other investigators will be able to accurately repeat the procedures that were actually used.

e. Interpreting Data – Experienced investigators try to have a clear idea about how data will be analyzed before any experiments begin. Because statistics are often used to help with data interpretation, knowing which statistical methods will be used can help decide how many replicate experiments or observations should be included in the experimental design. The observations and measurements made during an investigation usually do not turn out exactly as predicted, because nature is extremely complex and it is often impossible to control every important variable or even to predict all of the variables that may be important. Data may reveal problems with the experimental design or show that more data are needed to fully investigate the original hypotheses. On the other hand, few data may be needed to disprove a null hypothesis; for example, if the null hypothesis is that there is no difference between Group A and Group B, it only takes one observed difference to reject the null hypothesis. Of course, the scientific community would expect that subsequent investigators would also find differences between the two groups.

f. Drawing Conclusions – This is the moment of truth when the investigator decides whether the hypotheses have been supported or not. Often, it is not possible to draw firm conclusions from a single investigation; more data may be needed. Scientists almost never say that they have proven a hypothesis to be absolutely true, because they know that nature may behave differently at different places and at different times. Instead, they state their results in terms of probability: "Given a certain set of conditions, these are the results that we would expect in x percent of all observations."

4. Give each student group a copy of "Global Climate Change & Coral Recruitment: The Interactive Effects of Temperature and Ontogeny on the Biology of *Porites astreoides* Larvae. Mission Summary and Background." Tell students that their assignment is to develop one or more hypotheses related to Question #1, and to prepare an experimental design to test these hypotheses. Have each group prepare a report that:
   - States their hypothesis (or hypotheses);
   - Outlines their proposed experimental design;
   - Describes how variables will be controlled;
   - Identifies potentially important variables that cannot be controlled; and
   - Provides any necessary operational definitions.

5. Have each student group present their hypotheses and experimental designs to the entire class. These may take many different forms; the important thing is that students can articulate their ideas and explain a plausible line of reasoning that supports their approach. Hypotheses may postulate one or more specific differences between adult and larval corals (e.g., larvae are more sensitive to temperature change than adults) or may postulate that there are no differences between larvae and adults (null hypothesis). The investigators for this mission used the null hypothesis approach. At least one operational definition should describe the specific characteristic(s) that will be measured or observed to test the hypothesis.

   Given the broad potential effects of temperature on physiological processes, there are many possible characteristics that might be observed. Considering the information provided in the "Background" section, perceptive students may correctly infer that photosynthesis and respiration were characteristics that were observed in this investigation. Students are not expected to be familiar with instruments that could be used to make these observations (though this could be a suitable topic for independent research) but should understand that measuring the consumption or production of oxygen would provide a means of quantifying respiration and photosynthesis respectively.

Students should realize that this investigation will probably require some means of collecting larvae as they are released from the parent corals, and this is an opportunity to display their creativity in designing traps for the larvae. Researchers on this mission used control-top pantyhose that were held in place over coral colonies with a mesh collar. A glass centrifuge tube was attached to the opposite end of the pantyhose and collected the larvae as they left the parent corals.

There are a great many variables that might affect the outcome of this experiment, including:

- individual genetic variation among different coral colonies (controllable to some extent by doing experiments on larvae from a single colony, then repeating these experiments with larvae from different colonies);
- time of day (controllable to a degree by repeating experiments at different times of day);
- season or time of year (not controllable in a short-term investigation);
- presence of pollutants in the reef system (not really controllable, since the number of potential pollutants is very large and it is not practical to try to test for all of them); and
- human variability caused by investigators not performing exactly the same manipulations for each experiment (not absolutely controllable; investigators have to try to keep things as identical as possible for each experiment).

Students should be able to describe at least five such variables, and recognize that not all of them can be controlled.

The BRIDGE Connection

www.vims.edu/bridge/ – Click on "Ocean Science" in the navigation menu to the left, then "Ecology," then "Coral"

The "Me" Connection

Have students write an essay on a real or imaginary situation in which they might use the steps for designing a scientific investigation. The essay should include an appropriate hypothesis, and a short description of the experiments or observations that would be made.

Connections to Other Subjects

English/Language Arts

Evaluation

Individual data analyses and participation in group discussions provide opportunities for assessment.

Extensions

Visit http://www.uncw.edu/aquarius/ to learn about other *Aquarius* missions and activities.

Design a new technological tool that could be used to make measurements or observations to test hypotheses developed in Step #4.

3. Visit http://www.uncw.edu/aquarius/archive/2002/06_2002/profiles.htm and research one of the scientists that participated in this *Aquarius* mission. What was their role in the investigation? Why did these scientists need to live underwater to complete their research?

Resources

http://www.uncw.edu/aquarius/virtual_tour/ipix.html – Virtual tour of the *Aquarius* undersea laboratory http://www.marinebiology.org/science.htm – Odyssey Expeditions web site, with lots of information about coral reef fishes and reef ecology

http://www.reef.org/ – Reef Environmental Education Foundation web site

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard C: Life Science

- Interdependence of organisms

Content Standard D: Earth and Space Science

- Geochemical cycles

Content Standard E: Science and Technology

- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Natural resources
- Environmental quality
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

Content Standard G: History and Nature of Science

- Nature of scientific knowledge

Student Handout

Global Climate Change & Coral Recruitment: The Interactive Effects of Temperature and Ontogeny on the Biology of *Porites astreoides* Larvae

Peter Edmunds, Principal Investigator

Mission Summary and Background

Mission Summary

[from http://www.uncw.edu/aquarius/archive/2002/06_2002/expd.htm]

Over the next century it is predicted that the effects of global climate change will intensify, and the destructive effects on Earth's ecology will become more severe. Coral reefs provide one example of an ecosystem that is severely threatened by global climate change and rising sea-water temperatures that will result. Importantly, corals live close to their upper thermal limit and many coral reefs have already suffered substantially from the effects of human-caused destruction. In the next ten years, degraded reefs such as many in the Florida Keys, may suffer catastrophic losses from the combined effects of global warming and other stresses, from which they will be unable to recover. Despite research efforts and substantial public recognition that coral reefs are in crisis, surprisingly little attention has been paid to coral reproduction and the resulting coral larvae that are critical to reef maintenance and ultimately recovery. The goal of this project is conduct a multidisciplinary analysis of the biology, physiology, and genetics of coral larvae to understand how global warming will affect the coral population structure of reefs in the Florida Keys.

The first year of the project will use *Aquarius* to collect larvae from *Porites astreoides* (commonly and locally referred to as mustard hill coral due to its shape and color) and to carry out experiments to examine three questions:

1. Do adult and larval corals display physiological differences that might make the larvae more (or less) sensitive to changing temperatures? This is an important question as adult corals are stuck on the sea floor, and it is only their offspring —the larvae—that can drift through the water and find new (possibly better) places to live. Thus, it is important to know whether the larvae have a similar, or better, resistance to high temperatures than the adults, as the larvae likely will have to survive a wider range of conditions.

2. Does larval age and history affect their response to environmental challenges? Our earlier work in 1999 made the sur-

prising discovery that the larvae grow fast. More specifically, it seems that their physiology and behavior changes over periods of days, which might affect how they respond to different kinds of stress. For example, a hot day immediately after they are released may be more damaging than a hot day several days later. This portion of our research will help to understand how weather conditions during a period of coral reproduction might affect how many corals settle and grow on the reef.

3. Do the larvae and adults contain the same types of symbiotic algae? This is a very exciting question, and one that has become important since it was discovered that corals contain many different "types" or "strains" of symbiotic algae. Even scientists are not clear what "types" or "strains" refers to, but we are sure that the different types are at least as different as species are in other systems. Because the algae are different, there is the possibility that the larvae may contain algae that are different than those found in the parent. This could have a strong effect on how well the larvae survive after they are released from their parents and enter the water column. To answer this question, we will be collecting samples for the analysis of algal DNA that will help us to identify the different types found in adults and the larvae they release.

Background

[Based on a project proposal to the National Undersea Research Program by Peter Edmunds, Ruth Gates, and Ove Hoegh-Guldberg]

Over the last fifty years, coral reefs have experienced worldwide declines in coral cover that have been driven by a combination of natural and anthropogenic disturbances. It is likely that such declines will continue into the 21st century, particularly as global warming takes hold, sea temperature rises further, and coral bleaching becomes common. One scenario predicts that most coral reefs will experience annual bleaching by 2020, as a result of elevated seawater temperatures, a frequency of damage from which coral reefs may not be able to recover. Faced with the specter of profound change, there is intense interest in understanding the mechanisms by which corals respond to disturbances, and in assessing how (or whether) reefs can recover from collapses. The successful recruitment of juvenile corals is central to the persistence and/or recovery of coral reefs, and like most benthic organisms, the mortality, survivorship, and success of planktonic larvae will play a strong role in determining the distribution of juveniles. In corals, little is known about the processes governing larval success and thus it remains a formidable task to predict how, or to what extent, environmental perturbations, such as temperature disturbances, might affect larval survival and the trajectories of change in coral populations.

The free-living planula larvae of corals are the product of sexual reproduction, and only planulae have the potential to recruit to denuded areas and maintain genetic diversity (although asexual mechanisms such as fragmentation can also be important in some cases). In corals, the planula larval stage lasts from minutes to months depending on life history strategy, developmental rates, and ability to feed, and is concluded by a descent to the benthos and settlement. Larvae that settle and metamorphose have the potential to recruit into the adult coral population. Although coral planulae have been studied for more than a century, surprisingly few of these studies have examined the impact of environmental perturbations, although there is evidence that planulae are adversely affected by changes in salinity, temperature, light availability, and pollution. Given that adult corals live close to their upper thermal limit, elevated temperatures are likely to have a profound effect on planula physiology. Previous research has shown that when larvae of the “mustard hill coral” *Porites astreoides* are exposed to extreme temperatures, larval mortality and metamorphoses increase. One prediction of these results is that modest increases in temperature might increase coral recruitment (due to increased metamorphosis) but reduce larval survivorship. The net result for coral populations probably is negative, though, since the observed increase in mortality is considerably greater than the observed increase in recruitment.

Given the profound effects of elevated temperatures on enzymatic processes and the proximity of corals to their upper thermal limit, there is little doubt that increases in temperature will affect coral physiology. One result is likely to be the acceleration of respiration and the accompanying increased demand for oxygen, consumption of ATP, and depletion of carbon substrates. Increased temperature also affects photosynthesis, initially causing an increase, but then a decline as temperature reaches a critical level. The overall consequences of these changes to larvae are difficult to predict, because the response will vary depending on the temperature stress encountered, the types of zooxanthellae present, and the age of the larvae. Nevertheless, at extreme temperatures (e.g., 32°C) the combined effect of heightened respiration and depressed photosynthesis would be a rapid consumption of energy reserves that would likely reduce larval longevity and increase mortality.

Brooding corals (including *P. astreoides*) retain their larvae in the gastrovascular cavity for a time before releasing them to the external environment. In contrast, spawning corals release gametes into the external environment, and larval development takes place entirely outside the parent corals. The larvae released from brooding corals, or those that develop in the water from spawning species, can be competent to settle within a few days of release or fertilization respectively. At the time of settlement, mature larvae are different from earlier stages and have a well-developed ability to discriminate among substrata. Settlement starts a profound reorganization of the planula into a coral polyp. Thus, the lifespan of a planula larva includes profound developmental changes involving overall morphology, biochemistry, and numerous changes in gene expression. Any one of these processes could be affected by temperature stress with either immediate or long-term consequences. For example, temperature induced changes in larvae could affect the survival of newly-settled larvae, juvenile corals, or even adult corals. Our preliminary experiments suggest that the effects of temperature stress vary markedly with larval age.