Controlled experiments

How scientists conduct experiments and make observations to test hypotheses.

Introduction

Biologists and other scientists use the scientific method to ask questions about the natural world. The scientific method begins with an observation, which leads the scientist to ask a question. She or he then comes up with a **hypothesis**, a testable explanation that addresses the question. A hypothesis isn't necessarily right. Instead, it's a "best guess," and the scientist must test it to see if it's actually correct. Scientists test hypotheses by making predictions: if hypothesis XXXX is right, then YYYY should be true. Then, they do experiments or make observations to see if the predictions are correct. If they are, the hypothesis is supported. If they aren't, it may be time for a new hypothesis.

How are hypotheses tested?

When possible, scientists test their hypotheses using controlled experiments. A **controlled experiment** is a scientific test done under controlled conditions, meaning that just one (or a few) factors are changed at a time, while all others are kept constant. We'll look closely at controlled experiments in the next section.

In some cases, there is no good way to test a hypothesis using a controlled experiment (for practical or ethical reasons). In that case, a scientist may test a hypothesis by making predictions about patterns that should be seen in nature if the hypothesis is correct. Then, she or he can collect data to see if the pattern is actually there.

Controlled experiments

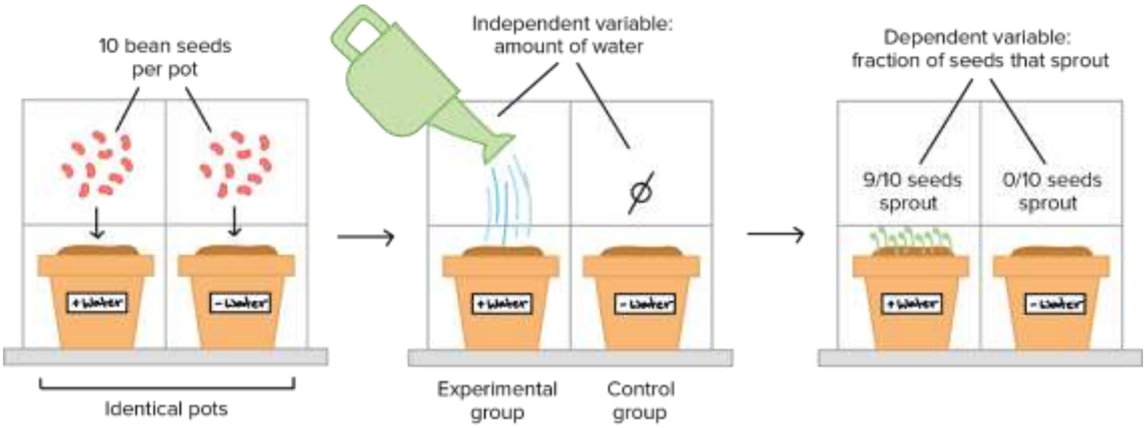
What are the key ingredients of a controlled experiment? To illustrate, let's consider a simple (even silly) example.

Suppose I decide to grow bean sprouts in my kitchen, near the window. I put bean seeds in a pot with soil, set them on the windowsill, and wait for them to sprout. However, after several weeks, I have no sprouts. Why not? Well...it turns out I forgot to water the seeds. So, I hypothesize that they didn't sprout due to lack of water.

To test my hypothesis, I do a controlled experiment. In this experiment, I set up two identical pots. Both contain ten bean seeds planted in the same type of soil, and both are placed in the same window. In fact, there is only one thing that I do differently to the two pots:

- One pot of seeds gets watered every afternoon.
- The other pot of seeds doesn't get any water at all.

After a week, nine out of ten seeds in the watered pot have sprouted, while none of the seeds in the dry pot have sprouted. It looks like the "seeds need water" hypothesis is probably correct! Let's see how this simple example illustrates the parts of a controlled experiment.



Panel 1: Two identical pots are prepared. 10 bean seeds are added to each pot. The pots are placed near the window.

Panel 2: One pot (experimental group) is watered. The other pot (control group) is not watered. The independent variable is the amount of water given.

Panel 3: In the experimental (watered) pot, 9/10 seed sprout. In the control (un-watered) pot, 0/10 seeds sprout. The fraction of seeds that sprout is the dependent variable.

Control and experimental groups

There are two groups in the experiment, and they are identical except that one receives a treatment (water) while the other does not. The group that receives the treatment in an experiment (here, the watered pot) is called the **experimental group**, while the group that does not receive the treatment (here, the dry pot) is called the **control group**. The control group provides a baseline that lets us see if the treatment has an effect.

Not necessarily. In general, a controlled experiment must always have a control group as a baseline. However, there may be several experimental groups, each with a slightly different treatment applied to it.

For instance, we could have a control group of bean seeds that got no water, plus three experimental groups: one that got water every day, one that got water every two days, and one that got water once a week.

Independent and dependent variables

The factor that is different between the control and experimental groups (in this case, the amount of water) is known as the **independent variable**. This variable is independent because it does not depend on what happens in the experiment. Instead, it is something that the experimenter applies or chooses him/herself.

Independent variables

Experimental results are much more straightforward to interpret and analyze when there is just one independent variable (one factor changed at a time). As a general rule of thumb, especially when you are starting out in biology, you should limit yourself to one independent variable per experiment.

Once you have lots of lab experience and some background in statistics, you can consider doing experiments with two independent variables at once. For example, you might want to see how water and light levels jointly affect bean seed sprouting. A well-designed experiment with two independent variables can tell you whether the variables interact (modify each other's effects). However, experiments with more than one independent variable have to follow specific design guidelines, and the results must be analyzed using a special class of statistical tests to disentangle the effects of the two variables.

Dependent variables

Having more than one dependent variable is pretty straightforward. To add a dependent variable, you simply pick another outcome you'd like to measure for each of the groups in your experiment. For instance, in addition to recording the fraction of sprouted bean seeds in each pot, we might also measure the height of the sprouts. In this case, both fraction of seeds sprouted and height of the sprouts would be dependent variables.

In contrast, the **dependent variable** in an experiment is the response that's measured to see if the treatment had an effect. In this case, the fraction of bean seeds that sprouted is the dependent variable. The dependent variable (fraction of seeds sprouting) *depends* on the independent variable (the amount of water), and not vice versa.

Experimental **data** (singular: *datum*) are observations made during the experiment. In this case, the data we collected were the number of bean sprouts in each pot after a week.

Variability and repetition

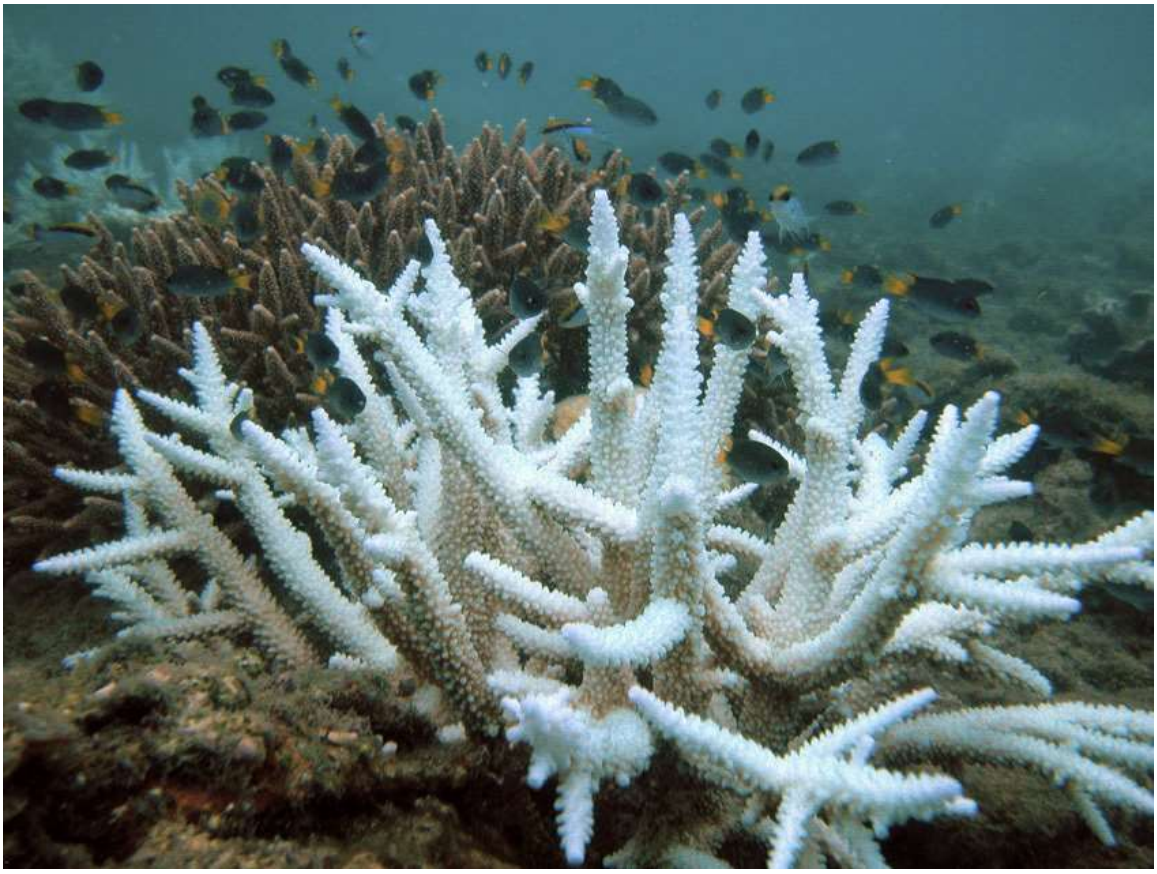
Out of the ten watered bean seeds, only nine came up. What happened to the tenth seed? That seed may have been dead, unhealthy, or just slow to sprout. Especially in biology (which studies complex, living things), there is often variation in the material used for an experiment – here, the bean seeds – that the experimenter cannot see.

Because of this potential for variation, biology experiments need to have a large sample size and, ideally, be repeated several times. **Sample size** refers to the number of individual items tested in an experiment – in this case, 10101010 bean seeds per group. Having more samples and repeating the experiment more times makes it less likely that we will reach a wrong conclusion because of random variation.

Biologists and other scientists also use to help them distinguish real differences from differences due to random variation (e.g., when comparing experimental and control groups).

Controlled experiment case study: CO^2

As a more realistic example of a controlled experiment, let's examine a recent study on coral bleaching. Corals normally have tiny photosynthetic organisms living inside of them, and bleaching happens when they leave the coral, typically due to environmental stress. The photo below shows a bleached coral in front and a healthy coral in back.



Photograph showing a bleached, white coral in the foreground and a healthy, brownish coral in the background.

Image credit: "[Keppelbleaching]" ([CC BY 3.0]).

A lot of research on the cause of bleaching has focused on water temperature1^11. However, a team of Australian researchers hypothesized that other factors might be important too. Specifically, they tested the hypothesis that high CO^2 which make ocean waters more acidic, might also promote bleaching2^22start superscript, 2, end superscript.

What kind of experiment would *you* do to test this hypothesis? Think about:

- What your control and experimental groups would be
- What your independent and dependent variables would be
- What results you would predict in each group

Have you given it a try?

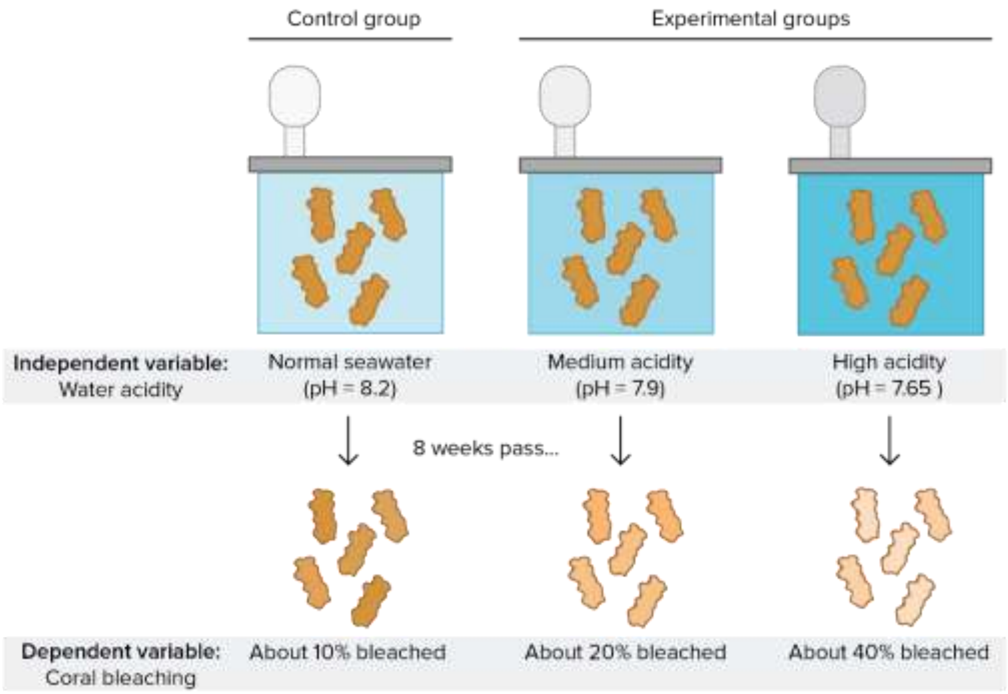
Experimental setup

The Australian team collected many fragments of a certain coral species (*Acropora intermedia*) from the Great Barrier Reef. Then, they split the fragments into three groups, putting each group in water with a different pH (acidity level). After eight weeks, the researchers checked each fragment to see how much it had bleached.

- Some corals were grown in tanks of normal seawater, which is not very acidic (pH around 8.2). The corals in these tanks served as the **control group**.
- Other corals were grown in tanks of seawater that were more acidic than usual due to addition of CO^2. One set of tanks was medium-acidity (pH about 7), while another set

was high-acidity (pH 7.65). Both the medium-acidity and high-acidity groups were **experimental groups**.

- In this experiment, the **independent variable** was the acidity (pH) of the water. The **dependent variable**, was the degree of bleaching of the corals. seawater.
- The researchers used a large sample size and repeated their experiment. Each tank held 5555 fragments of coral, and there were 5555 identical tanks for each group (control, medium-acidity, and high-acidity).



Experimental setup to test effects of water acidity on coral bleaching.

Control group: Coral fragments are placed in a tank of normal seawater (pH 8.2).

Experimental group 1: Coral fragments are placed in a tank of slightly acidified seawater (pH 7.9).

Experimental group 2: Coral fragments are placed in a tank of more strongly acidified seawater (pH 7.65).

The water acidity is the independent variable.

8 weeks are allowed to pass for each of the tanks...

Control group: Corals are about 10% bleached on average.

Experimental group 1 (medium acidity): Corals are about 20% bleached on average.

Experimental group 2 (higher acidity): Corals are about 40% bleached on average.

Degree of coral bleaching is the dependent variable.

Note: None of these tanks was "acidic" on an absolute scale. That is, the pH values were all above the neutral pH 7.0. However, the two groups of experimental tanks were moderately and highly acidic *to the corals*, that is, relative to their natural habitat of plain seawater.

Analyzing the results

When they analyzed their results, the researchers found that corals in the medium-acidity water lost about 20%, percent of their color on average, while those in the high-acidity water lost about 40%, percent of their color on average. The control samples, in contrast, lost just a little over 10%, percent of their color.

This example shows why it is important to have a control group: thanks to the control, the researchers knew that their corals bleached a little in normal water when grown in tanks, but bleached more in water that was extra acidic. Using statistical tests, the researchers found that acidity level had a significant effect on bleaching (that is, that the higher levels of bleaching in the experimental tanks were unlikely to be explained by random variation).

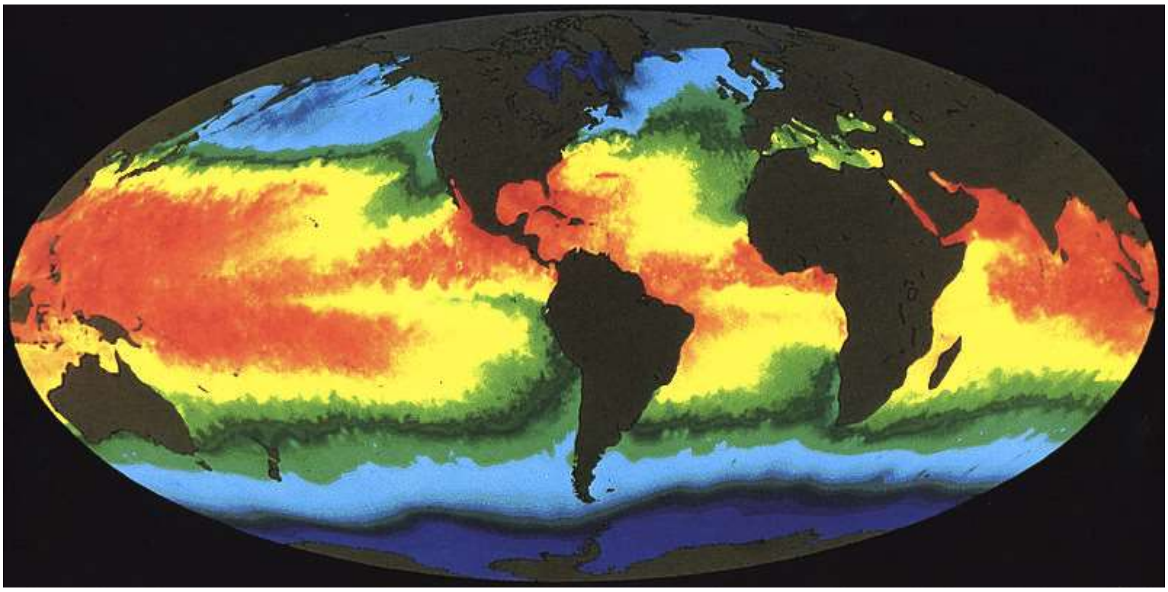
Non-experimental hypothesis tests

Some types of hypotheses can't be tested in controlled experiments for ethical or practical reasons. For example, a hypothesis about viral infection can't be tested by dividing healthy people into two groups and infecting one group: infecting healthy people would not be safe or ethical. Similarly, an ecologist studying the effects of rainfall can't make it rain in one part of a continent, while keeping another part dry as a control.

In situations like these, biologists may use non-experimental forms of hypothesis testing. In a non-experimental hypothesis test, a researcher predicts observations or patterns that should be seen in nature if the hypothesis is correct. She or he then collects and analyzes data, seeing whether the patterns are actually present.

Case study: Coral bleaching and temperature

A good example of hypothesis testing based on observation comes from early studies of coral bleaching. As mentioned above, bleaching is when corals lose the photosynthetic microorganisms that live inside of them, which makes them turn white. Researchers suspected that high water temperature might cause bleaching, and they tested this hypothesis experimentally on a small scale (using isolated coral fragments in tanks)3,[4] What ecologists most wanted to know, however, was whether water temperature was causing bleaching for lots of different coral species in their natural setting. This broader question could not be answered experimentally, as it wouldn't be ethical (or even possible) to artificially change the water temperature surrounding entire coral reefs.



False-colored map representing sea surface temperatures around the globe as different colours. Warmers colors, mostly near the equator, represent hotter temperatures, while cooler colours, mostly near the poles, represent cooler temperatures.

Image credit: "Global sea surface temperature," by NASA (public domain).

Instead, to test the hypothesis that natural bleaching events were caused by increases in water temperature, a team of researchers wrote a computer program to predict bleaching events based on real-time water temperature data. For example, this program would generally predict bleaching for a particular reef when the water temperature in the reef's area exceeded its average monthly maximum by $1^{\circ}C$ or more.

The computer program was able to predict many bleaching events weeks or even months before they were reported, including a large bleaching event in the Great Barrier Reef in 1998[1]. The fact that a temperature-based model could predict bleaching events supported the hypothesis that high water temperature causes bleaching in naturally occurring coral reefs.