Using Microbial Ecology to Teach Experimental Design and Sampling Methods

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Exercise 3: Student Handout
Considering Replication

It is essential to consider the design of an experiment before setting out to collect data. Faulty experimental design can make it impossible to draw meaningful conclusions regarding the hypothesis being tested and can result in a waste of time and resources—valuable commodities in scientific research. Before an experiment is conducted the following should be determined: what, if any, factors (variables) will be manipulated, how will the factors be manipulated, and what are the appropriate methods for sampling and data collection.

In some cases a scientific investigation involves comparing responses to variable sets of conditions. For example the effects of plant fertilizer on the abundance of nitrogen-fixing bacteria could be studied by applying different known concentrations of fertilizer to plots of agricultural land. Differences in the abundances of nitrogen-fixing bacteria before and after application of the fertilizer could be compared. The treatments in this experiment are the different concentrations of fertilizer. The control would be a plot of land that received no fertilizer. Good experimental design requires that the treatments and controls vary as little as possible in all factors except those that are manipulated, in this case fertilizer concentration. So for example, the experimental agricultural plots should vary little in the amount of water and sun they receive.

Where possible an experimental design should include replication. Replicates in an experiment should all be treated identically. They represent the natural background variation in a system. For example, a researcher interested in determining the number of viable bacteria in a body of water would not rely on counting bacteria once from a single sample of the water. Such an action could lead to faulty conclusions because that single sample of water might have fewer, or more, bacteria in it than another sample of the same volume. To account for such variance in the number of bacteria present it would be necessary to collect three, or preferably more, samples in identical fashion (replicate samples) and count the number of bacteria in each. These values would subsequently be used to calculate an average number of bacteria per unit volume of water for the habitat being sampled.

Statistics are used to evaluate the significance of differences detected between groups that receive variable treatment in an experiment. Most statistical calculations assume that replicate values are independent observations. This means that a single replicate value does not depend upon one other value more than it does on another. Each replicate should be treated the same as other replicates as much as possible. Using replicates that are not statistically independent is a form of pseudoreplication, a term coined by the ecologist Stuart Hurlbert in 1984 (1). Pseudoreplication also results from treating multiple measurements of the same sample as replicate measurements. An example is drawing multiple aliquots from a single flask of bacterial culture and referring to each

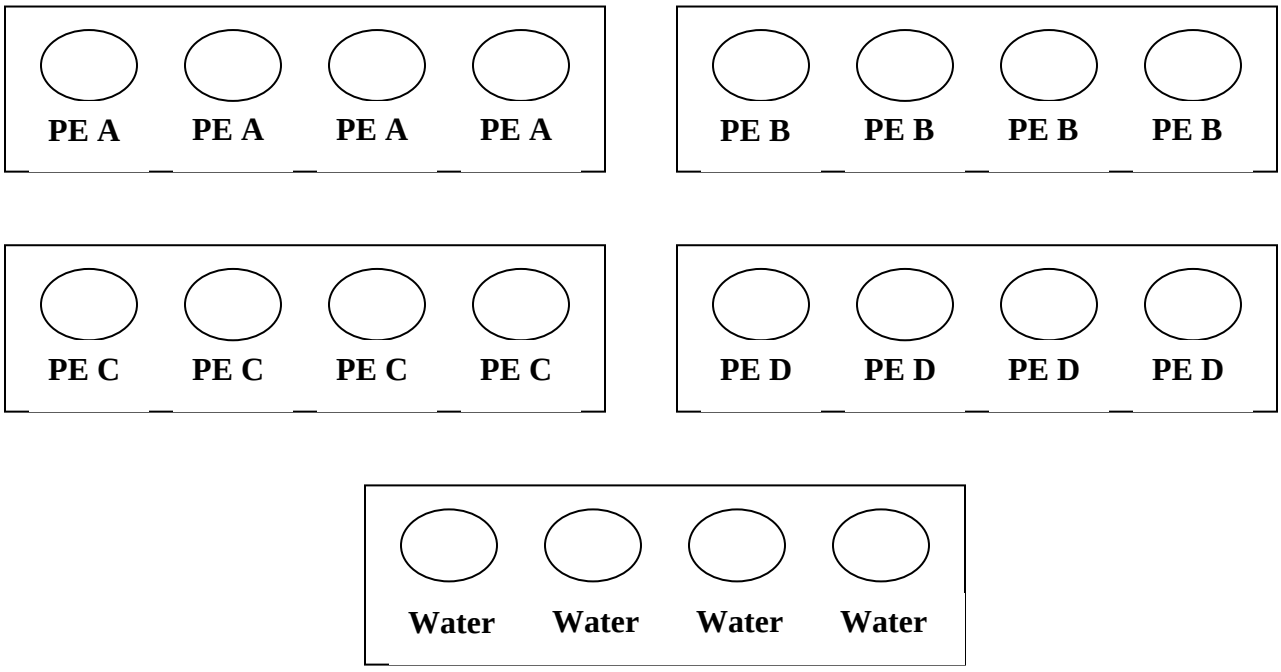
aliquot as a different replicate. An adequately replicated experiment would involve taking a single aliquot each from multiple flasks, all treated identically during the experiment. Pseudoreplication decreases our ability to draw accurate conclusions about what is happening in an experimental system. It does so by effectively lowering the sample size of an experiment and therefore decreasing chances that the natural background variation in a system will be adequately detected.

The series of word problems that follow is designed to help you think about the concept of replication in experimental design.

Part I.

A microbiologist is interested in testing how well extracts from three different plants (A, B, and C) can inhibit the growth of a single species of bacterium. The scientist plans to conduct her tests in shallow plastic wells. Into each well she will add equal volumes of plant extract and bacterial culture and incubate the cultures at the same temperature in a single incubator. She will compare growth after 24 hours by measuring turbidity in each of the wells using a spectrophotometer. She plans to replicate her experiment four times and sets it up as indicated in the diagram below. Each row of boxes indicates a single plastic dish, each of which contains four shallow wells.

PE refers to the plant extract solution taken from one of the three plants, A, B, or C. Water was used in place of a plant extract as a control.

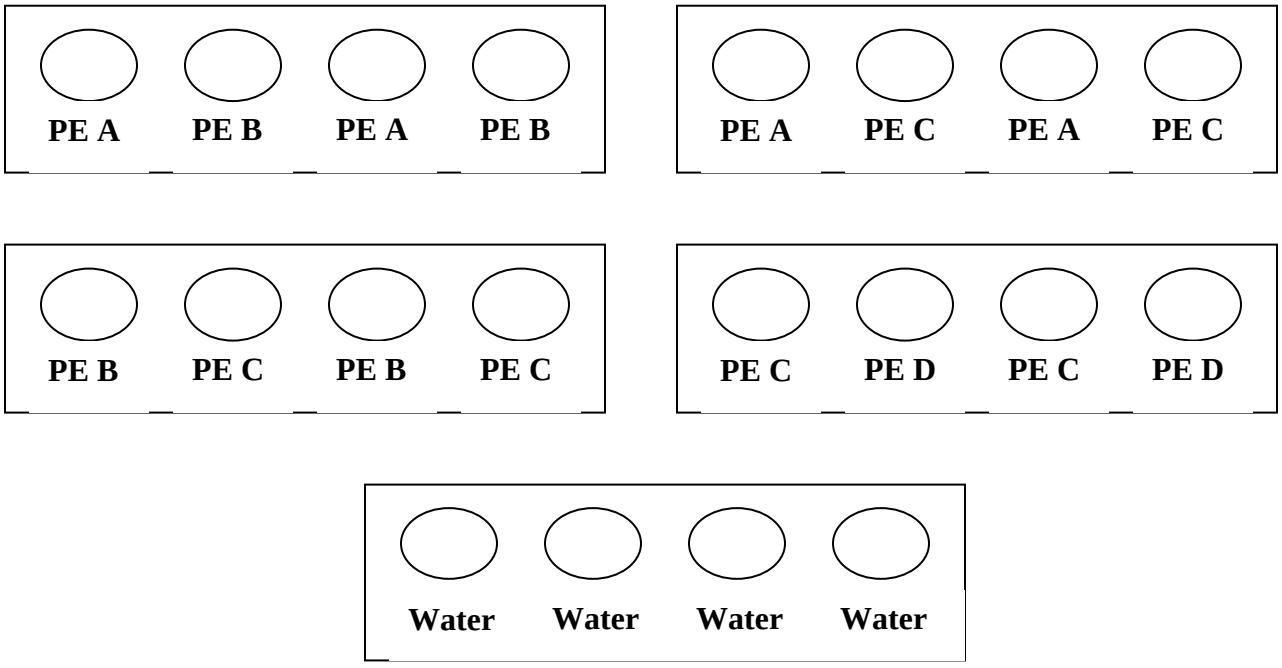


What constitutes a treatment in this experiment?

Is there some aspect of the experimental design that could result in the experimenter finding differences and falsely attributing them to the treatment (plant types) when they are really caused by something else? What, other than treatment level, could result in differences in bacterial growth between treatments in this experiment? Explain your answer.

Part II.

The experiment testing the effects of plant extracts on bacterial growth was redesigned to remove the effect that a plastic dish might have on differences in bacterial growth between treatments. The new design is illustrated below.



Does the new design adequately address the problems posed by the previous experimental design? Explain your answer.

Are the replicates of the plant extract treatments independent of one another? Explain your answer.

Think of a better way to replicate this experiment. You are not constrained to the materials used above. You may suggest other ways of setting up the experiment; be creative. Draw your design and explain why this is a better method.

Part III.

Wastewater from urban centers is normally processed through a local wastewater treatment facility where solids are removed and steps are taken to reduce numbers of bacteria in the water. Treated water is often emptied into a local body of water; so it is not uncommon to find wastewater treatment facilities located beside rivers. An aquatic microbiologist investigating a river system decides to investigate whether the number of bacteria in the river is influenced significantly by the outflow from a wastewater treatment facility. In particular, the scientist is interested in bacteria commonly found in human intestines, many of which are gram-negative, lactose-fermenting rods collectively referred to as coliform bacteria. The microbiologist plans to collect water samples, return them to the laboratory, collect the bacteria on filters, and grow only the coliform bacteria using media selective for their growth. The number of coliform bacteria isolated from different locations in the river can be compared. Your job is to help the microbiologist design the experiment up through the stage where water samples are filtered and plated for isolation of bacteria. Describe how samples should be collected and used so that uniformity amongst replicates is ensured and pseudoreplication is avoided. Also describe an approach to the experiment that results in pseudoreplication and explain why.

Reference.

1. **Hurlbert, S. H.** 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54:**187–211.