Using Microbial Ecology to Teach Experimental Design and Sampling Methods

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EXERCISE 3: INSTRUCTOR GUIDE
CONSIDERING REPLICATION

Part I.
This section should take approximately 10 minutes.

What constitutes a treatment in this experiment and how many different treatments are there? The treatments in this experiment are the different plant extracts (PE), so there are three treatments: PE A, PE B, and PE C.

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. Yes, if there are differences between individual plastic dishes that affect bacterial growth, this could result in measurable differences between groups of treatments. The experimenter could attribute the variability in results to effectiveness of plant extracts, when the variability could instead be due entirely, or in part, to differences in the individual plastic dishes. In other words the conclusions of the experiment would be false.

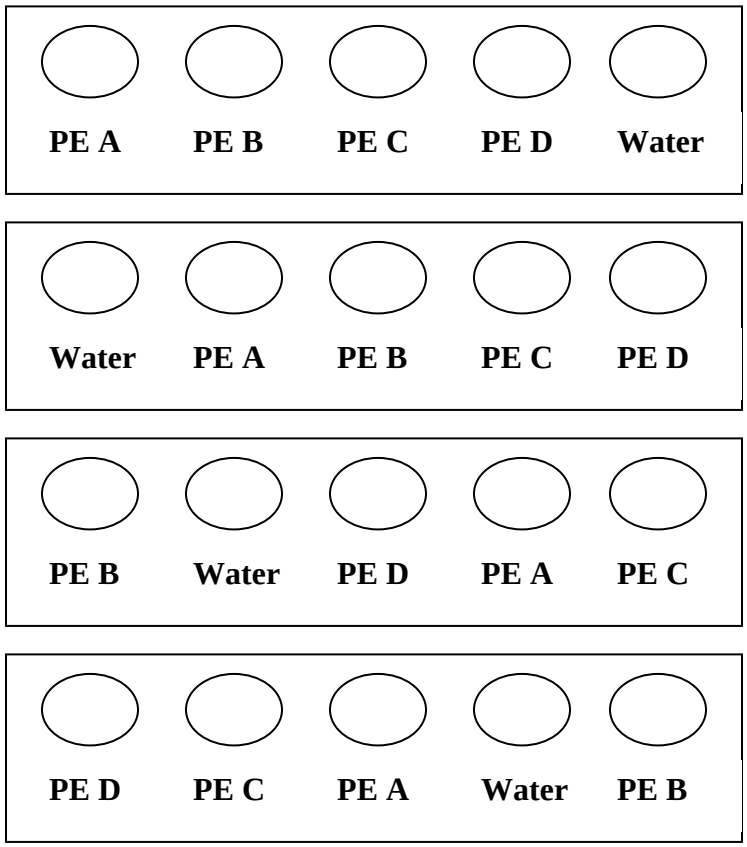
Part II.
This section should take approximately 15 minutes.

What constitutes a replicate in this experiment? The replicates are the four wells in a single plate. Each well in a plate contains exactly the same materials—extract from the same plant (A, B, or C) and bacteria—so they are each replicates of an experimental treatment.

Are the replicates of the plant extract treatments independent of one another? Explain your answer. No, the replicates are not independent of one another because they are not all treated the same. For example, the association of PE A with PE B in the same dish might influence the outcome of the experiment more than the association of PE A with PE C (together in a different dish). Therefore the replicates of PE A are not independent because one may depend more on its association with PE B than one not as closely associated with PE B.

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. Answers to this question will be variable. Students should take into consideration that all treatments and the control should experience the same set of conditions and that replicates should be independent. Below is an example of an experimental design that uses plastic dishes with

five wells. Each plastic dish contains all four treatments and a control, so the dishes themselves will not impact the results of one treatment more than another. The replicates are independent of one another because they are each randomly assigned to a spot in each dish. Students may suggest setting the experiment up in test tubes or flasks, in which case they should consider placement of these in a test tube rack or an incubator. The placement of different treatments and replicates should follow similar guidelines as those used to assign them to wells in the dishes below.



Additional notes. As part of the experimental design, students might also consider the source of the bacteria for the experiment. If all of the bacteria are taken from a single flask, this would constitute a form of pseudoreplication, where multiple measurements of the same sample are treated as replicate measurements. To adequately replicate the experiment, treatment wells should be inoculated from four different flasks of bacteria. Culture from one flask would be used to inoculate one set of wells of each treatment plus one control (one PE A well, one PE B well, etc.), while culture from a separate flask would be used to inoculate the second set of treatment and control wells, etc. This ensures that the results of the experiment are applicable to more than a single flask of bacteria. Especially motivated students might ask how many generations each flask of bacteria should be separated by to ensure they represent the true genetic variation of the bacterial population being investigated.

Part III.
This section should take approximately 20 minutes.

The task assigned to the students at the end of the experimental scenario is purposefully not specific. It is meant to encourage students to integrate what they have learned from Parts I and II with a more complete consideration of pseudoreplication. Students should be encouraged not to focus too much on the smallest details of the sampling and methods for isolating bacteria. Rather, they should focus their attention on a good experimental design. For students less familiar with experimental design and microbiology, it might be helpful to provide some leading questions. Examples include: From where should water samples be collected? How many samples should be collected from each site? What will constitute a replicate sample? How can statistical independence of these replicates be ensured?

The experiment can be designed in a variety of ways. Listed below are some considerations to ensure the question is addressed and replicates are uniform and independent.

- Water samples should be taken from a site at the wastewater treatment outflow and upstream of this site so that the influence of the outflow on numbers of antibiotic-resistant coliform bacteria can be compared to normal background levels. Students may also propose sampling downstream of the outflow, which is a good idea as it indicates how localized the effect of the outflow might be. The number of upstream and downstream sampling sites may vary; the final decision will depend on resources (time and supplies), but obviously more sampling sites allow the experimenter to draw broader conclusions.
- Once the location of sampling sites is determined, the number of samples collected from each site should be determined. These will ultimately provide the replicates of the experiment, so at least three should be collected, as this is the minimum number necessary to compute an average. Obviously, having more replicates is generally better.
- A single aliquot from each water sample should be filtered for collection and isolation of bacteria. The number of coliform bacteria isolated from this aliquot of water constitutes one replicate value. The average number of bacteria isolated from all of the replicates taken from a single site can be compared to an average value for a different site.
- A common pitfall is to suggest taking a single water sample from each site on the river, returning this sample to the laboratory and withdrawing multiple aliquots from it for filtration and isolation of bacteria. This is pseudoreplication because multiple measurements of the same sample are treated as replicate measurements, which they are not.