

5.2: Experimental units and sampling units

Introduction

Sampling units refer to the measured items, the focus of data collection; samples are selected from populations. Often, sampling units are the same thing as individuals. For example, if we are interested in the knowing whether men are more obese than women in Hawai'i, we would select individuals from the population; we would measure individuals. Thus, the sampling unit would be individuals, and the measurement unit would be percent body fat recorded for each individual. The data set would be the collection of all body fat measures for all individuals in the study, and we would then make inferences (draw conclusions) about the differences, if any, between adult males and females for body fat.

But sampling units can also refer to something more restrictive than the individual. For example, we may be interested in how stable, or consistent, a person's percent body fat is over time. If we take a body fat measure once per year over a decade on the same group of adults, then the sampling unit refers to each observation of body fat recorded (once per year, ten times for an individual), and the population we are most likely to be referring to is the collection of all such readings (ten is arbitrary — we could have potentially measured the same individual thousands of times).

In some cases the researcher may wish to compare groups of individuals and not the individuals themselves. For example, a 2001 study sought to see if family structure influenced the metabolic (glycemic) control of children with diabetes (Thompson et al 2001). The researchers compared how well metabolic control was achieved in children of single parents and two-parent families. Thus, the sampling units would be families and not the individual children.

Experimental units

Experimental units refers to the level at which **treatments** are independently applied in a study. Often, but not always, treatments are applied directly to individuals and therefore the sampling units and experimental units in these cases would be the same.

Question 1: What is the sampling unit in the following cell experiment?

A technician thaws a cryo tube containing about $\frac{1}{2}$ million A549 cells (Foster et al 1998) and grows the cells in a T-75 culture flask (the 75 corresponds to 75 cm² growing area) in a CO₂ incubator at 37 °C. After the cells reach about 70% confluency, which may represent hundreds of thousands of cells, the technician aliquots 1000 cells into twelve wells of a plate for a total of 12000 cells. To three wells the technician adds a cytokine, to another three wells he adds a cytokine inhibitor, and to another three wells he adds both the cytokine and its inhibitor, and to the last three wells he adds DMSO, which was the solvent for both the cytokine and the inhibitor. He then returns the plate to the incubator and 24 hours later extracts all of the cells and performs a multiplex quantitative PCR to determine gene expression of several target genes known to be relevant to cell proliferation.

The described experiment would be an example of a routine, but not trivial procedure the technician would do in the course of working on the project in a molecular biology laboratory.

The choices for numbers provided in the description we may consider for the number of sampling units are:

- A. 1000 cells
- B. 12,000 cells
- C. $\frac{1}{2}$ million cells
- D. 3 wells
- E. 12 wells
- F. The target genes
- G. None of the above

The correct answer is G, None of the above.

“None of the above” because the correct answer is ... there was just one sampling unit! All cells trace back to that single cryo tube.

To answer the question, start from the end and work your way back. What we are looking for is independence of samples and at what level in the experiment we find independence. Our basic choice is between numbers of cells and numbers of wells. Clearly, cells are contained in the wells, so all of the cells in one well share the same medium, being treated the same way, including all the way back to the cryo tube — all of the cells came from that one tube so this lack of independence traces all the way back to the source of the cells. Thus, the answer can't be related to cell number. How many wells did the technician set up? Twelve total. So, the maximum number of sampling units would be twelve, unless the samples are not independent. And clearly the wells (samples)

are not independent because, again, all cells in the twelve wells trace back to a single cryo tube. Thus, from both perspectives, wells and cells, the answer is actually just one sampling unit! (Cumming et al 2007; Lazic 2010). Finally, the genes themselves are the targets of our study — they are the variables, not the samples. Moreover, the same logic applies — all copies of the genes are in effect descended from the few cells used to start the population.

Experimental and sampling units often, but not always the same

Question 2: What is the experimental unit in the described cell experiment?

- A. 1000 cells
- B. 12,000 cells
- C. ½ million cells
- D. 3 wells
- E. 12 wells
- F. The target genes
- G. None of the above

The correct answer is E, 12 wells. Noted above, the technician applied treatments to 12 wells. There were two treatments, cytokine and cytokine-inhibitor (Table 5.2.1).

Table 5.2.1. Experiment description translated to a table to better visualize the design.

Well	DMSO	Cytokine	Cytokine inhibitor
1	Yes	Yes	No
2	Yes	Yes	No
3	Yes	Yes	No
4	Yes	No	Yes
5	Yes	No	Yes
6	Yes	No	Yes
7	Yes	Yes	Yes
8	Yes	Yes	Yes
9	Yes	Yes	Yes
10	Yes	No	No
11	Yes	No	No
12	Yes	No	No

Replication: Groups and individuals as sampling units

The correct identification of levels at which sampling independence occurs is crucial to successful interpretation of inferential statistics. Note the replication in Table 5.2.1: three cytokine, three cytokine-inhibitor, three with both. Sampling error rate is evaluated at the level of the sampling units. Technical replication of sampling units allows one to evaluate errors of measurement (e.g., instrument noise) (Blainey et al 2014). Replication of sampling units increases statistical power, the ability to correctly reject hypotheses. If the correct design reflects sampling units are groups and not individuals, then by counting the individuals as the independent sampling units would lead the researcher to think his design has more replication than it actually does. The consequence on the inferential statistics is that he will more likely reject a correct null hypothesis, in other words, the risk of elevated type I error occurs ([Chapter 8 – Inferential Statistics](#)). This error, confusing individual and group sampling units, is called **pseudoreplication** (Lazic 2010).

Consider a simpler experimental design scenario depicted in Figure 5.2.1: Three different water treatments (e.g., concentrations of synthetic progestins, Zeilinger et al. 2009) in bowls A, B, and C; three fish in bowl A, three fish in bowl B, and three fish in bowl C. The outcome variable might be a stress indicator, e.g., plasma cortisol (Luz et al 2008).

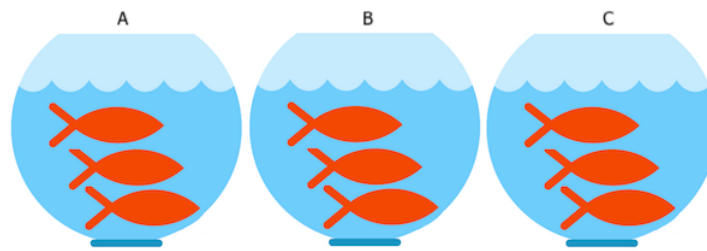


Figure 5.2.1: Three aquariums, three fish. Image modified from <https://www.pngrepo.com/svg/153528/aquarium>

Question 3: What were the experimental units for the fish in the bowl experiment (Fig. 5.2.1)?

- A. Three bowls
- B. Nine fish
- C. Three water treatments

The correct answer is A, 3 bowls. The treatments were allocated to the bowls, not to individual fish. The three fish in each bowl provides technical replication for the effects of bowl A, bowl B, and bowl C, but does not provide replication for the effects of the water treatments. Adding three bowls for each water treatment, each with three fish, would be the simplest correction of the design, but may not be available to the researcher because of space or cost limitations. The design would then include nine bowls and 27 fish. If resources are not available to simply scale up the design, then the researcher could repeat the study several times, taking care to control **nuisance variables**. Alternatively, if the treatments were applied to the individual fish, then the experimental units become the individual fish and the bowls reduced to a **blocking effect** (Chapter 14.4), where differences may exist among the bowls, but they are no longer the level by which measurements are made. Note that if pseudoreplication is present in a study, this may be accounted for by specifying the error structure in a linear mixed model (e.g., random effects, blocking effects, etc., see Chapter 14 and Chapter 18).

Question 4: What were the sampling units for the fish in the bowl experiment (Fig. 5.2.1)?

- A. Three bowls
- B. Nine fish
- C. Three water treatments

The correct answer is B, the individual fish. If instead of aqueous application of synthetic progesterin, treatments were applied directly to each fish via injection, what would be the answers to Question 3 and Question 4?

Choices like these clearly involve additional compromises and assumptions about experimental design and inference about hypotheses.

Conclusion

Sampling units, experimental units, and the concept of level at which units are independent within an experiment were introduced. Lack of independence yields the problem of pseudoreplication in an experiment, which will increase the chance that we will detect differences between our treatment groups, when no such difference exists!

Questions

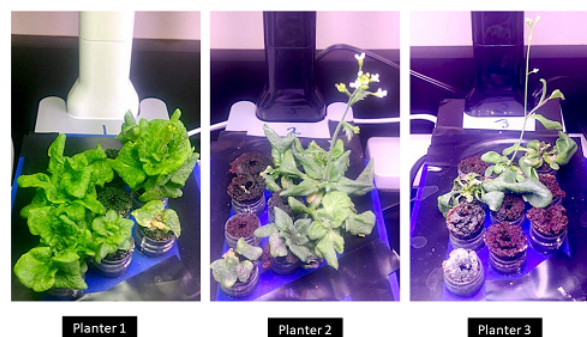


Figure 5.2.2: Three Miracle-Grow AeroGarden planters, each with nine seedlings of an *Arabidopsis thaliana* strain.

1. Nine seeds each of three strains of *Arabidopsis thaliana* were germinated in three Miracle-Grow AeroGarden® hydroponic planters (Fig. 5.2.2). Each planter had nine or ten vials with sphagnum peat. All seeds from a strain were planted in the same apparatus, one seed per vial. What were the experimental units?

- A. planters
- B. seeds
- C. strains of *Arabidopsis*
- D. vials in the planters

2. This experimental design is an example of pseudoreplication, but at what level?

- A. planters
- B. seeds
- C. strains of *Arabidopsis*
- D. vials in the planters

3. How would you re-do this experiment to avoid pseudoreplication? (Hint: you can't add more planters!)

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