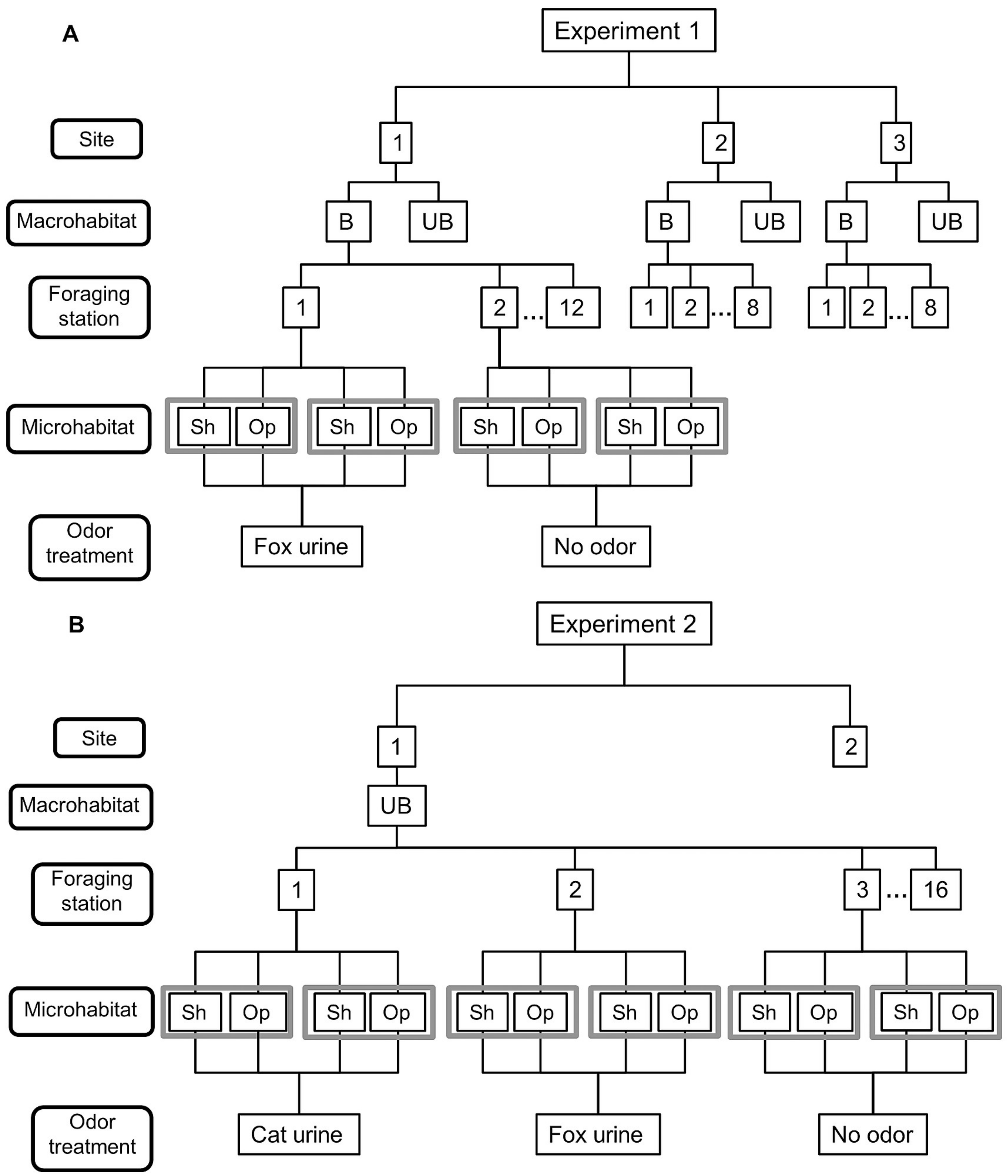
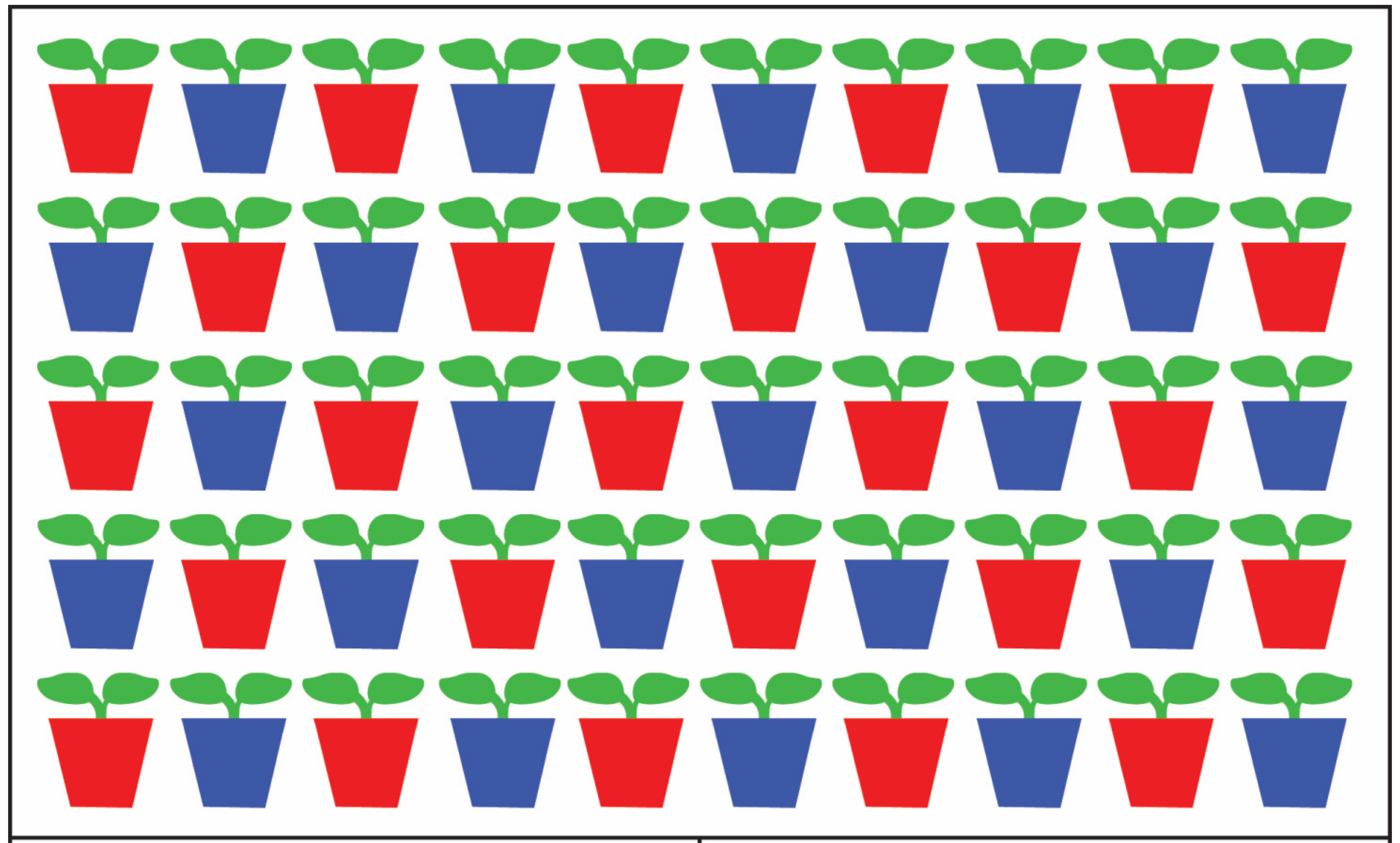
Foundational Statistics

Study / Experimental Design





One Measurement Per Pot	Three Measurements Per Pot
Total Sample Size (N) = 50	Total Sample Size (N) = 150
Experimental Units = 50	Experimental Units = 50
Treatments = 2	Treatments = 2
Treatment Size (n)= 25	Treatment Size (n)= 25
Replication (r) = 25	Replication (r) = 25

Key terms used in experimental design

Term	Definition	Example
Measurement	A single piece of recorded information reflecting a characteristic of interest (e.g. length of a leaf, pH of a water aliquot mass of an individual, number of individuals per quadrat etc)	Protein content of the milk of a single female koala
Observation	A single measured sampling or experimental unit (such as an individual, a quadrat, a site etc)	A small quantity of milk from a single koala
Population	All the possible observations that could be measured and the unit of which wish to draw conclusions about (note a statistical population need not be a viable biological population)	The milk of all female koalas
Sample	The (representative) subset of the population that are observed	A small quantity of milk collected from 15 captive female koalas[a]
Variable	A set of measurements of the same type that comprise the sample. The characteristic that differs (varies) from observation to observation	The protein content of koala milk.

Key terms used in experimental design

Variable	A set of measurements of the same type that comprise the sample. The characteristic that differs (varies) from observation to observation	The protein content of koala milk.

Dependent Variable: ("response variable" or "*y*-variable")
The variable(s) you are trying to make inferences about, by measuring its "response" to other variables.

Independent Variable: ("predictor variable" or "predictor" or "*x*-variable")
You are trying to understand whether/how these influence the dependent variables.

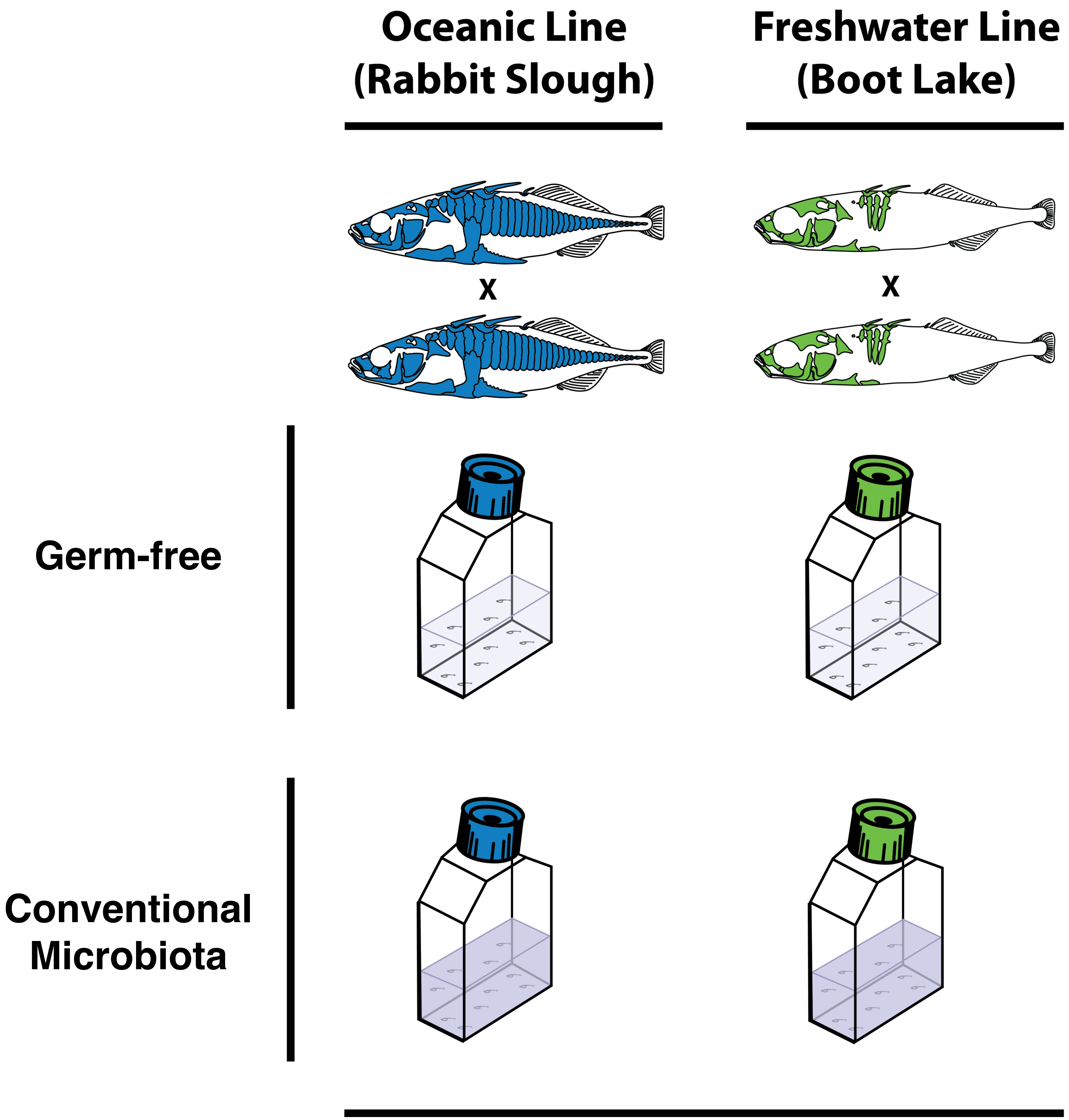
A biological example to get us started

- EXAMPLE - Say you perform an experiment on two different strains of stickleback fish, one from an ocean population (RS) and one from a freshwater lake (BP) by making them microbe free. Microbes in the gut (in particular) are known to interact with the gut epithelium in ways that lead to a proper maturation of the immune system.

- EXPERIMENTAL SETUP - You decide to carry out an experiment by treating multiple fish from each strain so that some of them have a conventional microbiota, and some of them are not inoculated with any bacteria (germ-free). You then measure the levels of gene expression in the stickleback gut using RNA-seq. Because you have a suspicion that the sex of the fish might be important, you track it too.

- PLANNING ANALYSIS - Do this before the actual experiment! What are the properties and dimensions of the variables, and why does it matter? Are there “unplanned variables?”

A biological example to get us started



Most important study design concepts

Replication - Larger samples (at the appropriate level) give parameter estimates with less uncertainty (i.e. smaller standard error).

$$\sigma_{\bar{x}} \approx s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

Randomization - We model our data after random variables. Our samples and assumptions about sampling distributions should emulate "random draws of observations."

Independence - Observations within a sample should be independent of one another except for the experimental factor(s) that we are varying to study. "Confounding" factors are associations (non-independence) that make it seem like our independent variables are the sole influences, when actually they are not.

What is an experimental study?

- In an *experimental study* the researcher assigns treatments to units or subjects so that differences in response can be compared.
  - Clinical trials, reciprocal transplant experiments, factorial experiments on competition and predation, etc. are examples of experimental studies.

- In an *observational study*, nature does the assigning of treatments to subjects. The researcher has no influence over which subjects receive which treatment.

Common garden “experiments”, QTL “experiments”, etc, are examples of observational studies.

What is an experimental study?

- In an experimental study, there must be at least two treatments
- The experimenter (rather than nature) must assign treatments to units or subjects.
- The crucial advantage of experiments derives from the <u>random assignment of treatments to units</u>.
- Random assignment, or randomization, minimizes the influence of confounding variables, allowing the experimenter to isolate the effects of the treatment variable.

Clinical Trials

- The gold standard of experimental designs is the clinical trial. Experimental design in all areas of biology have been informed by procedures used in clinical trials.

- A clinical trial is an experimental study in which two or more treatments are assigned to human subjects.

- The design of clinical trials has been refined because the cost of making a mistake with human subjects is so high.

- Experiments on nonhuman subjects are simply called "laboratory experiments"or "field experiments", depending on where they take place.

Design components of **controlled experiments** such as clinical trials

- The goal of experimental design is to eliminate bias and to reduce sampling error when estimating and testing effects of one variable on another.
- To reduce bias, the experiment included:
  - Simultaneous control group: the study included both the treatment of interest and a control group (the subjects receiving a placebo).
  - Randomization: treatments are randomly assigned to subjects at each clinic.
  - Blinding: neither the subjects nor the clinicians knew which subjects were assigned which treatment.

- To reduce the effects of sampling error, the experiment included:
  - Replication: the study was carried out on multiple independent subjects.
  - Balance: the number of subjects was nearly equal in the two groups at every clinic.
  - Blocking: subjects were grouped according to the clinic they attended, yielding multiple repetitions of the same experiment in different settings ("blocks").

Simultaneous control group

- In clinical trials either a placebo or the currently accepted treatment should be provided. A placebo is an inactive treatment that subjects cannot distinguish from the main treatment of interest.

- In experiments requiring intrusive methods to administer treatment, such as injections, surgery, restraint, or confinement, the control subjects should be perturbed in the same way as the other subjects, except for the treatment itself, as far as ethical considerations permit. The “sham operation”, in which surgery is carried out without the experimental treatment itself, is an example.

- In field experiments, applying a treatment of interest may physically disturb the plots receiving it and the surrounding areas, perhaps by trampling the ground by the researchers. <u>Ideally, the same disturbance should be applied to the control plots.</u>

Randomization

- Once treatments are chosen, the researcher should *randomize* assignment to units or subjects.

- Randomization means that treatments are assigned to units at random, such as by flipping a coin. Chance rather than conscious or unconscious decision determines which units end up receiving the treatment of interest and which receive the control.

- A *completely randomized design* is an experimental design in which treatments are assigned to all units by randomization.

Randomization

- Randomization breaks the association between possible confounding variables and the explanatory variable, allowing the causal relationship between the explanatory and response variables to be assessed.

- Randomization doesn't eliminate the variation contributed by potentially confounding variables, only their correlation with treatment.

- It ensures that variation from confounding variables is similar between the different treatment groups.

Randomization

- Randomization should be carried out using a random process, for example:
  - List all *n* subjects, one per row, in a computer spreadsheet.
  - Use the computer to give each individual a random number.
  - Assign treatment A to those subjects receiving the lowest numbers and treatment B to those with the highest numbers.

Experimental unit								
Random number	11	18	87	55	76	70	90	4
Treatment	A	A	B	A	B	B	B	A

- Other ways of assigning treatments to subjects are almost always inferior because they do not eliminate the effects of confounding variables.
- "Haphazard" assignment, in which the researcher chooses a treatment while trying to make it random, has repeatedly been shown to be non-random and prone to bias.

Stratified assignment vs. randomization