Instructions for Instructors:

Learning Objectives:

- Apply scientific method.
- Create hypotheses.
- Design experimental method using adequate controls and replication.
- Understand the factors that promote and inhibit plant growth.
- Use previous knowledge of statistics to know which statistical test is appropriate to analyze their data.
- Present data to a broad audience.

Materials required for each group:

- 1 tray (either a greenhouse plant or a lunch tray will be fine—just make sure it can catch excess water)
- 12 pots (or as many as will fit on the trays you have)
- Masking tape and sharpies to label pots
- 1 bale of soil per 8 groups (you can purchase this online or at any store)
- Large bin to mix soil in (this will need to be saturated before being put into pots)
- Growing station
    - We used shelves equipped with grow lights and connected those to timers so they would be on for 12hrs and off for 12hrs
- Watering can and spray bottles filled with water
- Seeds of the following species (I purchased them on amazon, but you can get most of these at any gardening store)
    - Clover
    - Alfalfa
    - Oats
    - Rye
    - Corn
    - Millet
    - Sorghum
    - Sunflower
    - Green beans
    - Or purchase more! I would recommend fast-growing crop species for this lab (I've had students use native prairie species in the past, but these don't grow quite fast enough for them to get good data).

General instructions:

- Have students come up with a question they want to ask about plant growth (based on the traits you present them with) or the environmental conditions they want to re-create, and THEN look at the species spreadsheet

    - Double check that they have selected species that do not differ in more than one way, unless they are deliberately testing the interaction between those things.
- Students should use maximum of 12 pots (4 treatments with 3 replicates of each, for example). **No fewer than 2 replicates for each treatment (double check that they have adequate replication before continuing)**
- Have students label each pot with masking tape, and label their tray with their section and group # (most of these will look identical for the first few weeks)
- Make sure students randomize the pots in the trays (lighting, temperature, and other factors might affect the treatments if they don't do this
- Students will fill pots to the brim with soil (pack it down with their hands). Then they need to completely saturate the soil BEFORE planting. (Students can wear gloves if they don't want to touch dirt, but remind them that getting your hands dirty is a great part of science!)
- All seeds should be planted slightly beneath the surface (half an inch or so) EXCEPT oats and millet, which should be surface sown (but pressed gently into the top of the soil)
    - **If students are using millet or oats, they MUST come in twice a week for the first week to spritz their pots with water from a spray bottle otherwise seeds will not germinate.**
- If students are growing both oats and millet, have them grow them in separate pots (difficult to tell apart). If this is not possible, you will have to help them sort through their plants and help tell which is which.
- General ideas (Do not need to be specific, but a good ballpark estimate) on # of seeds for being grown alone (not in competition experiment), as an indicator species, and as a competitor (4 treatments). If they want to do low/high competition, pick the second and fourth numbers. First number refers to total # of seeds planted, "cut to" indicates final number of plants to end up with 1wk after germination (so 2wks into experiment). removing extras beyond that number.

Species	**Alone**	**Indicator**	**Competitor**
Clover	35 (cut to 30)	15 (cut to 8)	0, 20, 45, 60
Alfalfa	35 (cut to 30)	15 (cut to 8)	0, 20, 30, 40
Oats	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Rye	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Corn	15 (cut to 10)	5 (cut to 3)	0, 5, 10, 15
Millet	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Sorghum	15 (cut to 10)	5 (cut to 3)	0, 10, 15, 20

- Watering: Students will need to come in once a week for the first week, twice a week for the second week, and three times for the third and fourth week. Before watering, students should press pinky finger into soil (just covering the nail). If nail comes out clean, water! If nail comes out damp, no need to water.

- If students want to add specific amounts of water, tell them to just say "watered until completely saturated" which means that water is dripping out the bottom of the pot". If students want to do a drought experiment (or manipulate the amount of water). Tell them to figure out the # of mL's of water required to completely saturate the pot (before seeds are added). 75% of that number should be well-watered. 25% for low water, and 50% for a medium treatment if they want that.
- General timeline:
  - **Week 1:** Come up with research question, design experiment and choose species. Begin to look through the primary literature to start to develop hypotheses.
  - **Week 2:** Plant seeds and continue developing hypotheses. Write up methods section.
  - **Week 3**: Check on plants, conduct measurements (if applicable), consider what statistics students will conduct on collected data.
  - **Week 4:** Check on plants, conduct measurements (if applicable), write up introduction and data analysis methods.
  - **Week 5:** Check on plants, conduct measurements (if applicable). Write R script to analyze data (if applicable). Design excel spreadsheet to input data into (if not created already) while also keeping in mind how it will be analyzed.
  - **Week 6:** Collect final biomass data, enter it into excel and analyze it. Begin designing research poster.
  - **Week 7:** Clean up, finish poster design.
  - **Week 8:** Present poster to class.

Student Instructions:

Why do we care about the environment?

While humans have learned to modify our environments (e.g., building houses with heating and air conditioners) many other organisms must rely on adequate environmental conditions, that over time, they have evolved to survive in. Importantly, the environment is rapidly changing, and organisms cannot evolve fast enough to keep up with these changing environmental conditions. This includes being exposed to warmer temperatures, drier climate, or environmental degradation. Two primary environmental factors in which an organism must adapt can be broken down into two categories: **biotic** and **abiotic** factors.

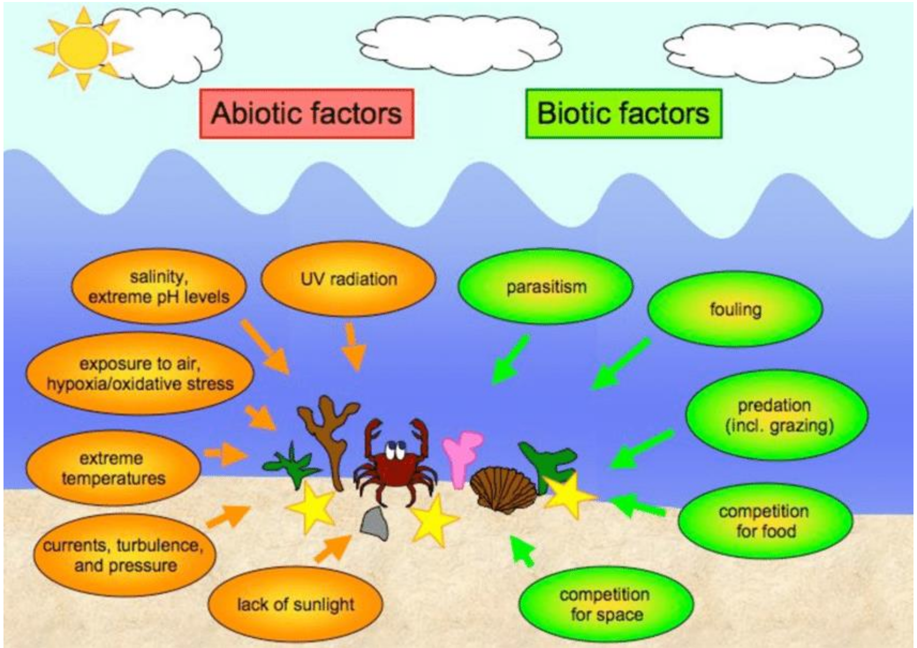

Figure 1: A diagram of which biotic (green) and abiotic (orange) factors can influence organismal survival in an aquatic ecosystem. Credit: Marc F Diederich

Biotic factors (or living things in an environment) are those factors that effect how the same species (**intraspecific**) or different species (**interspecific**) interact and affect each other within their environment. The presence of one species that eats another species (e.g., like the presence of brine shrimp in environments where anemones are present) makes an environment suitable for that organism. If two organisms that occupy the same habitat rely on a similar resource, and that resource is in limited supply, then there could be competition between organisms. Competition may involve **direct interactions** such as combat among organisms (e.g., the release of toxic chemicals in plants (e.g., allelopathy)) or **indirect interactions** such as making the environment less hospitable to others organisms (e.g., beavers flood a habitat which can change the microbiotic community).

Abiotic factors (or nonliving things in an environment) are resources such as light, water, nutrients (e.g., phosphorus and/or nitrogen), and space. If these resources are in adequate supply within the environment, you can expect that organisms will have high growth, survival, and reproduction. However, not all organisms require the same amount and type of these resources. For example, you would expect that plants in a tropical rainforest would require a very humid environment to survive, whereas cacti in the deserts of the southwest could survive for weeks without rain.

Determining what combination of abiotic and biotic factors can influence the fitness of an organism is an important area of research. Riley is currently researching how the traits plant species have are influenced by their environment, and how we can use our understanding of those traits to create healthy plant communities. **For group projects**

this semester, you will manipulate either (or both!) abiotic and biotic factor(s) to understand the influence those factors have on the growth and/or survival of plants.

Plants as a model system:

Of all the organisms on earth, plants are incredibly sensitive to their environment, and that's because they can't move! (Well, can't move much, at least). Once a plant begins to grow, it must adapt to its environment in order to survive. Most plants (~85%) use C3 photosynthesis, but some have evolved adaptations that allow them to live in a variety of environments. Some grasses have a C4 photosynthetic pathway, allowing them to photosynthesize more efficiently in hot, dry environments (Check out this video to understand the difference between those pathways). Others (such as legume plants) can live in low-nitrogen environments (Check out this video which explains the importance of Nitrogen to plants) by having a symbiotic bacteria (*Rhizobium*) in root nodules. These bacteria convert atmospheric nitrogen into a form utilizable by the plant. Other plants persist in high-competition environments by releasing chemicals that suppress the growth of other plant species, without affecting the growth of their own species (Check out this video for a more in-depth explanation of allelopathy). Importantly, these processes have trade-offs: Plants that do C3 photosynthesis can usually grow faster than plants that do C4 photosynthesis (As long as conditions favor the C3 pathway). Therefore, not all adaptations are useful in all environments--can you think of environments where Nitrogen-fixing bacteria may not be a great advantage to plants?

We also know that plants do not often exist in a monoculture, but are actually competing against other plants! To examine the effects of competition, you can compete two species against one another using, an **indicator species,** and a **competitor species**. If you keep the density of species i constant and vary the density of species j, you will be able to measure the effect of species j on species i. This type of experiment is referred to as an **additive design** because competitors are added to indicators so that the total density of the mixture increases (Harper, 1977).

The effect of intraspecific competition in plant populations can be examined by planting one and/or multiple species over a range of densities. For all density-dependent tests, the most common result is that the mean weight per plant decreases as density increases (left graph in Figure 2) such that the total yield (weight per area) approaches a constant value (right graph in Figure 2). You can think of this total weight per area as a carrying capacity (the number, or mass, of individuals that can be supported by a particular environment at any one time). Graphs such as Figure 2 are not only relevant to ecology and are often used in agriculture to estimate optimal planting density for a particular crop – the density beyond which total yield no longer increases.

Five grass species and two legume species will be available for experimentation (Table 1). Consider the specific adaptations of each species (growth form, seed size, nutrition, and photosynthetic pathway) as you design your experiments.

Table 1: Species to be used in competition experiments. Data includes whether a plant has bacteria to convert nitrogen (legume), whether it used C3 or C4 photosynthesis (pathway), whether it can produce chemicals to reduce plant survival (Allelopath), and the size of the seedlings (plant size).

Family	Species	Legume	Pathway	Allelopath?	Plant size
Fabaceae	Red clover	yes	C3	No	small
	Alfalfa	yes	C3	No	small
	Green bean	Yes	C3	No	tall
Poaceae	Common Oats	No	C3	No	medium
	Perennial rye	No	C3	Yes	small
	Corn	No	C4	No	tall
	Millet	No	C4	No	medium
Asteraceae	Sunflower	No	C3	Yes	tall

Table 2: Species and number of seeds to be used in competition experiments. This ensures that plants needed for the experiment germinate for density trials. Values are inflated to compensate for seeds that may not germinate. This is applied to species being tested alone (no competition), as an indicator species, or trial numbers within a four-trial competition or a two trial low/high competition experiment (pick the second and fourth numbers). Once germinated numbers can be "cut back" to desired numbers needed for experiment.

Species	Alone	Indicator	Competitor
Clover	35 (cut to 30)	15 (cut to 8)	0, 20, 45, 60
Alfalfa	35 (cut to 30)	15 (cut to 8)	0, 20, 30, 40
Green Bean	15 (cut to 10)	5 (cut to 3)	0, 5, 10, 15
Oats	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Rye	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Corn	15 (cut to 10)	5 (cut to 3)	0, 5, 10, 15
Millet	25 (cut to 20)	8 (cut to 5)	0, 10, 20, 30
Sorghum	15 (cut to 10)	5 (cut to 3)	0, 10, 15, 20

Here is a list of supplies we can currently offer you:

- Shade cloth (to reduce light availability)
- Spray bottles (to reduce water availability)
- Nitrogen fertilizer
- 16oz pots (Smaller pots may prevent growth)
- Soil (garden mix, but other mixes may be available upon request

Below a few questions you could answer (feel free to come up with something totally different!):

- What environmental factors increase or decrease organism survival?
- What density of individuals permits coexistence? (minimize competition)
- What density of individuals prevents coexistence (maximize competition)
- Do environmental factors influence any of these interactions? (Can you include any covariates in your model

Activity Instructions:

A. ***Designing your experiment -*** Student groups will design and conduct experiments to test hypotheses about the interaction between two likely competitors (interspecific competition) , or 1 plant species at different densities (intraspecific competition) Each experiment will consist of **3 density treatments**. A single treatment consists of one indicator species and one competitor species grown in a single pot. Each treatment will be replicated three times. Therefore, you will need **12 pots** for your group's experiment. As a response variable, you will be measuring at least plant biomass but you can consider monitoring other parameters that you think will be interesting.

B. Planting Procedure

For two-species experiments:

a. Each group will prepare (12) 3.5 inch pots for their experiment. Use tape to label each pot with indicator name, competitor name, competitor density (0, 10, and 30), replicate number, and lab section.

b. Remember that there should be 3 density treatments with 2 replicates each (12 pots total). Fill each pot with soil to the inner rim.

c. Since not all seeds will germinate, you should plant 3 indicator seeds in each pot; plant 0, 13, and 35 competitor seeds in appropriate **pots unless you have red clover**, then you should plant 0, 20, and 60. We will thin the plants down to 2 indicators and 0, 10, and 30 competitors next week.

d. Place the three seeds for the indicator in the center of each plot with a toothpick in the middle. Plant the competitor's seeds evenly across the soil surface.

e. Plant seeds at a depth of 2 times the seed size (except oats, and perennial rye should be planted on the soil surface. Place seeds on the surface and gentle pat them down.)

f. Put all the pots for your groups experiment in a plastic tray. Label the tray with the indicator, competitor, lab section, and group name. Place a dome overtop.

g. Now your plants will grow in their current conditions for three weeks.

For one-species experiments

a. Each group will prepare (12) 3.5 inch pots for their experiment. Use tape to label each pot with seed density (10, 20, 30, and 40), replicate number, and lab section.

b. Remember that there should be 4 density treatments with 3 replicates each (12 pots total). Fill each pot with soil to the inner rim.

c. Since not all seeds will germinate, you should plant 13, 23, 33, and 43 competitor seeds in appropriate pots **unless you have red clover**, then you should plant 0, 20, 40 and 60. We will thin the plants down to 0, 10, 20 and 30 competitors next week.

d. Plant seeds at a depth of 2 times the seed size (except oats, and perennial rye should be planted on the soil surface. Place seeds on the surface and gentle pat them down.)

e. Put all the pots for your groups experiment in a plastic tray. Label the tray with lab section, and group name. Place a dome overtop.

f. Now your plants will grow in their current conditions for three weeks.

Part 2: Weighing your plants

Data collection

1. Count the number of surviving indicators and competitors in each pot and record the data in the worksheet.

2. Carefully cut each plant at the soil, removing the indicators first.

3. Measure the wet weight of the aboveground biomass of all the indicators from a single pot.

4. Repeat for the competitor (if applicable).

5. Repeat this procedure for all pots and record data in spreadsheet.

6. Put plant material in compost bin.

Data analysis of competition experiment:

1. Calculate mean indicator wet weight for each pot (weight divided by number of plants).

2. Calculate the reciprocal mean plant weight (1/w) for the indicator species in each pot using the wet weight.