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Laboratory Aquaponics: Bringing Fish Farming, Gardening, and Miniature Biospheres into the Everyday Classroom

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This lab introduces the use of an aquaponic system to teach ecology concepts and practical applications of aquarium fish keeping and maintaining a garden. Aquaponics is a method of keeping/farming aquatic animals (e.g., fish, aquatic invertebrates) and vegetables (or fruits) simultaneously, using an inter-connected system designed to utilize nitrogenous wastes generated by aquatic animals to fertilize plants growing in a soil-less growing bed. In this lab, students learn how to collect weekly data on the growth of the red kidney bean plant (*Phaseolus vulgaris*). As extensions, instructors may use an ecosphere, which can serve as a model of a biosphere. This work was supported by an ABLE 2012 Roberta Williams Laboratory Teaching Initiative Grant.

Keywords: Aquaponics, Gardening, Ecosphere, Fish, Farming, Hydroponics, Aquaculture

Link to Supplemental Materials: http://www.ableweb.org/volumes/vol-37/park/supplement.html

Introduction

Aquaponics is the practice of raising fish (or other aquatic animals) and vegetables (or fruits) simultaneously in a recirculating system. It combines the fields of hydroponics (the practice of growing plants without soil but with water and regulation of dissolved nutrients) and aquaculture (the practice of growing animals or plants, such as fish, mollusks, or seaweeds, using a controlled outdoor or indoor aquatic environment). A typical aquaponic system (Fig. 1) includes an aquarium (for aquatic animals) physically connected to a growing bed (for plants). A pump is used to draw water from the aquarium into the growing bed and a drain empties this water back eventually into the aquarium. Every aquaponic system utilizes bacteria species that convert ammonia produced by the fish to nitrites then nitrates (different bacteria species perform each conversion) which are taken up by the plants for growth and maintenance. Accumulation of nitrogenous wastes is toxic to fishes, but as the plants use these chemical compounds, water is filtered before it returns to the aquarium. Thus, plants in the growing bed provide a form of filtration in addition to existing chemical, biological, and mechanical filtration.

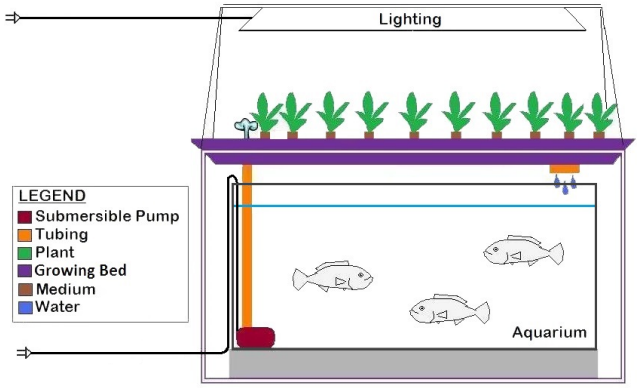


Figure 1. Generalized Flood-and-Drain Aquaponic System.

Types of Aquaponic Systems

A variety of aquaponic systems exist, which include but are not limited to Flood-and-Drain, Constant Height-

One Pump or CHOP (*sensu* Murray Hallam), or Barrel-ponics® (see Bernstein, 2011). The Flood-and-Drain is the simplest design, with the growing bed placed above an aquarium; water from the aquarium is pumped into the growing bed, which drains water back into the aquarium (see Fig. 1). In a CHOP design, a sump is incorporated between the growing bed and aquarium. In the typical CHOP system, the pump is in the sump. As water drains from the growing bed into the sump, the pump pumps this water into the aquarium, which has a drain near its top. As water fills in the aquarium, it drains automatically into the growing bed, and thus, the water level in the aquarium remains constant (unlike in the previous design), which is less stressful on the fish. Barrelponics® is a series of aquaponic systems originally designed by Travis Hughey. Their general design consists of a flood tank, growing bed, and aquarium, and the system overall operates similarly to a toilet. The pump is in the aquarium, and water is pumped into the head tank (also called flood tank), which sits above both the growing bed and aquarium. As aquarium water fills the flood tank, a valve will eventually go from closed to open, allowing water to enter the growing bed, which in turn eventually empties water into the aquarium. Please note that many variations exist for each of the generalized designs discussed above. The system used in the present work is a Flood-and-Drain system.

Best Practices for Growing Fish and Plants Together

In general, experienced aquaponics users recommend a 1:1 ratio of aquarium water-to-growing bed media (Rakocy *et al*., 2006; Bernstein, 2011). Our aquarium can hold up to 40 gallons (approximately 150 liters) of water but our growing bed can hold maximally just over 10 gallons (approximately 40 liters) of media. Thus, our system is not constructed for optimal plant yield. Furthermore, it is recommended that maintaining a fish stocking ratio of 1lb (approximately 450g) of mature fish per 5-20 gallons of aquarium water is manageable. For reference, an edible 30 cm tilapia typically weighs about 1.5lbs (700 g) (Bernstein, 2011). Thus, under ideal conditions, our aquarium can be used to raise somewhere between 2-5 edible-sized tilapia. Under commercial aquaculture conditions, tilapia grow, on average, 1g per day during the initial 90 days and 2.5g per day subsequently (Soderberg, 2006; Amadori, 2012).

Nitrification

In a new aquarium, "beneficial" bacteria must be established to neutralize nitrogenous waste chemicals. Fish produce ammonia as a waste product during excretion. Ammonia can also accumulate during decay of organic material. Too much ammonia makes aquarium water toxic and potentially fatal to fish. Over time, ammonia gets oxidized in an aquarium by *Nitrosomonas* bacteria which converts ammonia to the less toxic nitrite. As populations of *Nitrosomonas* grow, the concentration of ammonia eventually declines while the concentration of nitrite rises. Another bacteria, *Nitrobacter,* converts nitrite to nitrate (but see Hovanec *et al*., 1998). Nitrite concentration begins to decline as *Nitrobacter* populations grow, which coincides with increase in nitrate concentration. Nitrates can be readily used by plants and thus, is a major fertilizer of plants grown in an aquaponic system. The time it takes to establish adequate *Nitrosomonas* and *Nitrobacter* populations in an aquarium varies depending many factors (e.g., volume of water, amount of food given to fish), but professional aquarists usually recommend 2-3 months as a reasonable time frame to complete nitrification, which is also often referred to, in the aquarist community, as the completion of "cycling." (As an alternative, commercially available nitrifying bacteria can be purchased to expedite this process.) Thus, a new aquarium requires fish (or other aquatic organisms) that generate ammonia to kick start this "cycling." However, early on, ammonia levels must be regularly monitored to prevent abrupt "ammonia spikes" that may unexpectedly kill fish (or other aquatic organisms). Generally weekly or bimonthly 20% "water changes" (i.e., water removal followed by replenishment with new water) can help minimize the occurrence of ammonia spikes; 100% water changes should never be done while you have an aquarium because a complete overhaul of the existing aquarium water with new water (especially if you are using tap water that contains chlorine) will eliminate the very bacteria you are trying to culture. In summary, a newly constructed aquaponic system is unlikely to generate enough nitrates to foster optimal plant growth. Thus, care and time must be taken to establish mature populations of *Nitrosomonas* and *Nitrobacter* bacteria before growing a high load of plants in the growing bed.

Aquarium Filtration

Aquarium filtration typically comes in three forms: mechanical, chemical, and biological, all of which are usually included in a single electrically-powered filter. Mechanical filtration involves the removal of visible debris (e.g., eroded material, fine particles) and/or feces. Many electrically-powered filters draw aquarium water through a sponge, mesh, or fabric to physically remove this type of waste. Much of this waste must be removed before it decays and causes changes to water quality (i.e., accumulation of ammonia) to the aquarium water that may be harmful to fish. Chemical filtration involves the removal or dissolved wastes (e.g., heavy metals, organic molecules). This is usually achieved by running aquarium water through granular, porous carbon. Dissolved wastes get trapped in the pores, which prevents adversely altering the chemical composition of the aquarium water. Biological filtration involves the culturing of bacteria that carry out nitrification, which neutralizes and prevents the

accumulation of toxic nitrogenous waste molecules produced during excretion or decay. These bacteria can be found on any submerged surfaces within an aquarium. In an aquaponic system, such bacteria also grow in and on the growing bed media. Filters often include a section or structures (e.g., Bio-Filter Balls) that has large surface area to enhance growth of populations of these bacteria. Within filters, aquarium water flows over these surfaces continuously to maintain healthy cultures of these bacteria (also see **Nitrification** section).

Anatomy of the Red Kidney Bean Plant

The red kidney bean (*Phaseolus vulgaris*) plant is an herbaceous annual plant of the Fabaceae (legume) family, representatives of which can acquire nitrogen with a mutualistic relationship with nitrogen-fixing bacteria within root nodules (Gurevitch *et al*., 2002; Evert & Eichhorn, 2013). Red kidney bean plants are important in agriculture because of its edible seeds/beans (fruits). This plant species will be the central focus of our lab activity. Five arbitrarily-established "stages" of the red kidney bean plants have been identified to give students some experience in identifying key developmental features during the study period (Fig. 2). Stage I includes emergence of hypocotyl and roots but no visible leaves. Stage II is marked by appearance of unifoliate leaves located between the cotyledons but no extensive growth between the unifoliate leaves and the cotyledon (or cotyledon scar, if cotyledons no longer present) - in other words, there is no epicotyl. Stage III includes stem growth between the cotyledons (or cotyledon scar, if cotyledons no longer present) and unifoliate leaves - in other words, an epicotyl is present; unifoliate leaves are usually fully unfolded/spread open. Stage IV has at least one set of fully unfolded/spread-open trifoliate leaves in addition the unifoliate leaves. Stage V plants have trifoliate leaves, flowers, *and* fruits.

Student Learning Goals

Students will understand engineering principles and biological processes underlying an aquaponic system. More specifically, students will integrate knowledge of trophic cascade, nitrification, respiration, and photosynthesis.

Students will collect data on and analyze patterns of growth in red kidney bean plants grown for at least three weeks in an aquaponic system and a non-aquaponic system.

Students will appreciate the differences between continuous variables and categorical variables.

Student Outline

Preparation for Experiment (2-4 Days Before Week 0):

1. Germination of red kidney beans in preparation for potting. The instructor has prepared at least twice as many beans as needed for the experiment to ensure adequate sample sizes during experiment setup. To achieve germination, beans were soaked in fresh water for at least one hour and then placed between moist paper towels in a tray. (We have found that the red kidney beans we use may germinate as early as two days after soaking.) Check daily that paper towels are moist and clean.
2. Check that all equipment in the aquaponic system is functioning properly, especially timers, check valves, and lighting. Students should check that all the plumbing that connects the growing bed and aquarium is free of obstruction and thus will drain properly.

Week 0: Setup (No Measurements) (Total Time: 30-45 Min)

1. Each student (or pair of students) must acquire at least three (3) 3.5" netted pots for the aquaponic system and three (3) 3.5" standard potting soil pots for the non-aquaponic (soil) system. Label each pot with student initials, date, and a unique specimen number.
2. Fill netted pots completely with clay pebbles and rinse with tap water. Fill potting soil pots with soil. Students should randomly select a sprout and place it gently into the center of the medium within each pot, no deeper than 1 cm from surface.
3. Place each netted pot into the aquaponic system growing bed container. Place each standard potting soil pot into the non-aquaponic (soil) growing bed container.
4. Adjust timer settings: The aquaponic system's submersible pump must be set at a 90 min on : 90 min off alternating cycle. The fluorescent lighting must be set at a cycle of 12 hour light : 12 hour off for both the aquaponic system and non-aquaponic (soil) system. Be mindful that plants in the non-aquaponic (soil) system must watered periodically.

Students will measure the following traits every week:

Weeks 1-3: Measurements (Total Time: 30-45min Per Day)

Students will complete each of the following tasks (minimally once per week, on the same day and time of the week). Measurements should be acquired for both the aquaponic system and non-aquaponic (soil) system and written on the attached datasheet.

1. Determine the "stage" of each bean plant (Fig. 2). Mark this on your datasheet.

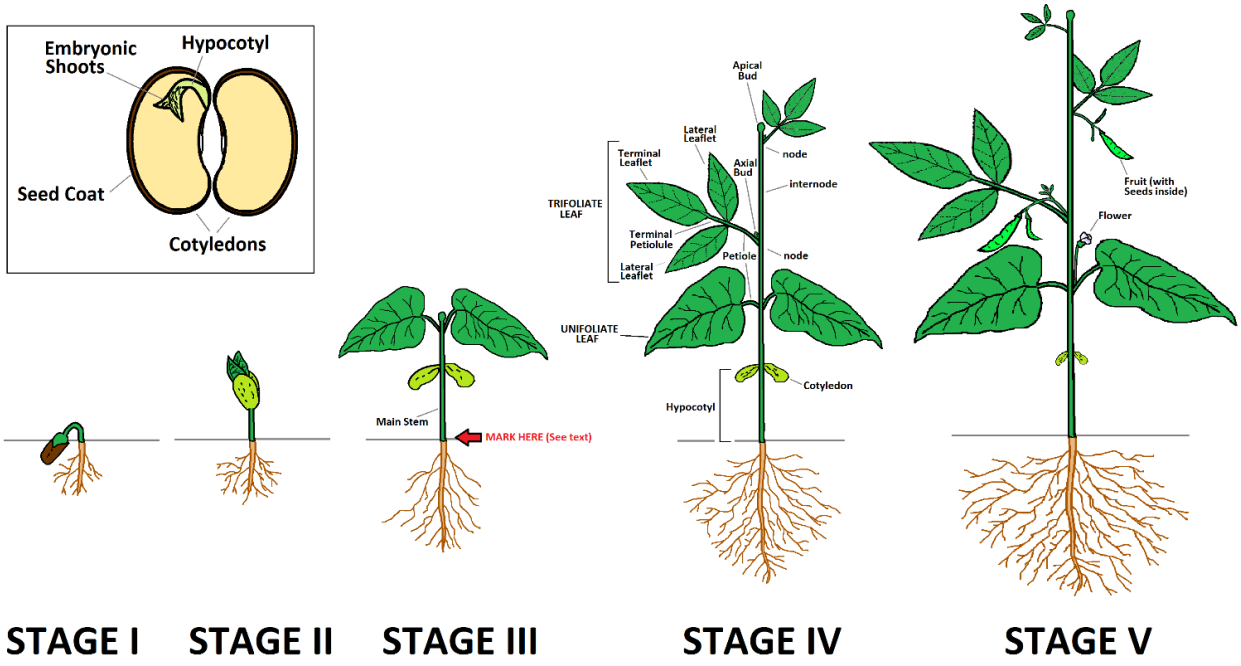


Figure 2. Putative life stages of red kidney bean (*Phaseolus vulgaris*) plant.

2. Before taking any measurements during a given week, count the number of cotyledons (or cotyledon scars) that remain on the plant. If a cotyledon falls off, a scar remains.
3. Record the following character states. All length traits should be measured in metric units.
   a) *Main Stem Trunk Diameter*: Students must first mark this structure during Week 1 (Fig. 2 red arrow; see Fig. 3). To do so, carefully straighten the main stem of the plant below the cotyledons and mark, using a dark-colored marker (e.g., permanent marker), a line parallel and as close to the surface of the growing bed medium or soil. Measure the main stem trunk diameter at this mark. Marking the main stem trunk must be done only during Week 1. This line will be used as a reference to measure main stem trunk diameter, plant height, and hypocotyl length during this and all subsequent weeks.

   b) *Plant Height:* Carefully straighten the full length of the plant height (Fig. 3A). The basal end is the marked main stem trunk (see previous step). The terminal end or "top" of the plant is the base of the apical bud. This bud may or may not have developed trifoliate leaves. If the apical bud does not have leaves, the base of the bud should be used as the terminal end (Fig. 3B). If the apical bud does have leaves, the base of the petiole of that trifoliate leaf should be treated as the terminal end.

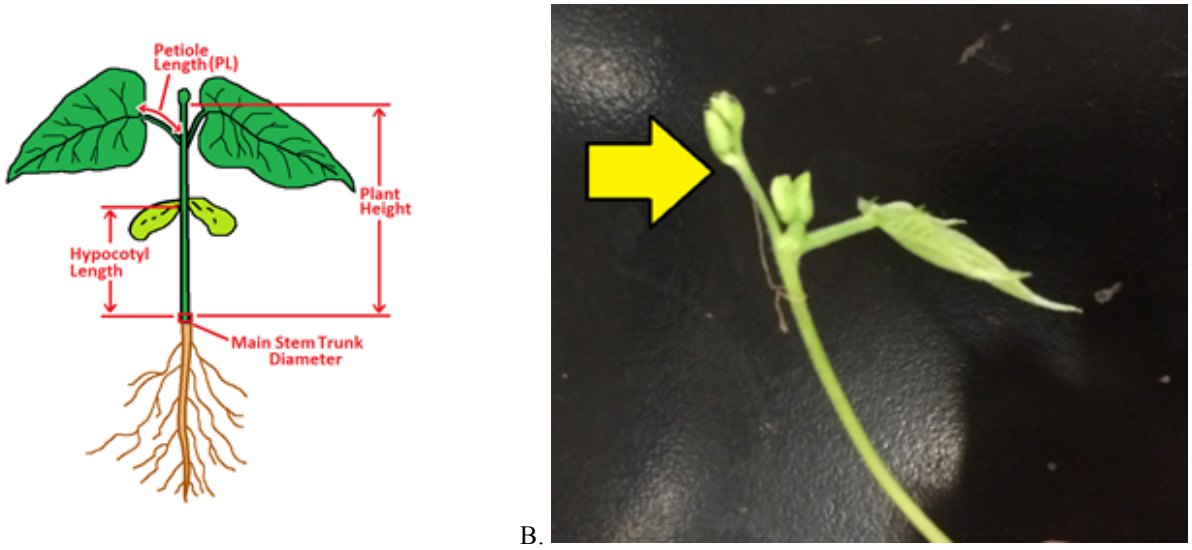


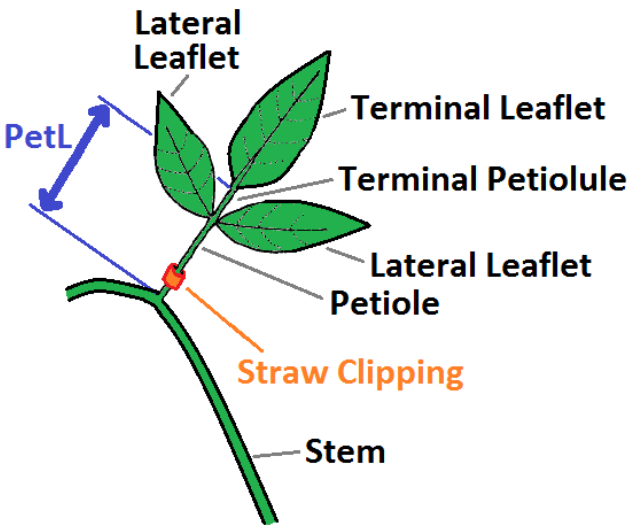
Figure 3. Plant Height and Associated Traits. A. Types of measurements on red kidney bean (*Phaseolus vulgaris*) plant, including of main stem trunk diameter, hypocotyl length, and length of unifoliate leaf petiole length. Position of base of main stem trunk must be marked with a marker. B. Base of apical bud (arrow).

   c) *Hypocotyl Length:* Carefully straighten the full length of the plant height. The basal end is the marked main stem diameter (see *Main Stem Trunk Diameter*). The terminal end of the hypocotyl is the location of the cotyledons (or cotyledon scars) (Fig. 3).
   d) *Leaf Petiole (+ Petiolule) Length = Lf_PetL*: Each leaf must be marked using a straw clipping; this step is only done once for a given leaf during the experiment. Prepare **straw label clippings** to mark each leaf petiole. Obtain a plastic straw. Cut out a small (3-5 mm) cross-section of the straw and then cut it lengthwise, forming a small ring that can be fastened to a stem. Mark "1" on this straw piece using a permanent marker. Wrap this clipping around a unifoliate leaf petiole. Make another straw label clipping, mark it "2," and wrap it around a different leaf petiole. Do the same (make a clipping labeled "3" for the third leaf petiole, and so on) for every remaining leaf petiole. Each unifoliate leaf petiole gets its own straw clipping. For trifoliate leaves, the base of each set of trifoliate leaves must get only one straw clipping (i.e., one straw clipping per trifoliate leaf, not per leaflet, see Fig. 4). For organizational and convenience purposes, it is preferred that all students use the same color straw for a given week and a different color for different weeks.
      (i) *Unifoliate Leaf (Simple Leaf) Lf_PetL:* Record petiole length. The unifoliate leaf is a simple leaf. In red kidney bean plants, they are the first two leaves to develop and grow during germination. Typically, they look heart-shaped and there are no more than two, in total, per plant. The petiole length of a unifoliate leaf is the distance between the base of the leaf and the node connected to the main stem of the plant (Fig. 3).

(ii) *Trifoliate Leaf (Compound Leaf) Lf_PetL*: Record sum of petiole + terminal petiolule length. A trifoliate leaf is a compound leaf, which is a leaf subdivided into separate leaflets, connected at their base by a petiole (Bell & Bryan, 2008). Trifoliate leaves develop later than unifoliate leaves, and a single trifoliate leaf consists of a set of three leaflets. The petiole of a trifoliate leaf is the distance between the base of the lateral leaflets and the node connected to the main stem of the plant. The petiole is usually longer than the terminal petiolule (NOTE: the terminal petiolule spans the base of the lateral leaflets and the terminal leaflet, which is the central separate leaflet, Fig. 4). The two lateral leaflets, that surround the terminal leaflet, do not have substantial petiolules and thus, their petiolules are not measured.

Sometimes, it may be difficult to determine when a "trifoliate leaf" should be classified as such when it is still in its early stages of emerging from a bud (see Fig. 4B). We use the rule of thumb that if an emerging petiole is less than 3 mm (i.e., less than the smallest straw clipping we can make and hang without damaging the leaf) and its leaflets are curled, then the developing trifoliate leaf is NOT counted as a measurable leaf.

A.



B.

Figure 4. Measuring "Lf_PetL" (sum of petiole + terminal petiolule lengths) of a trifoliate leaf. A. Diagram of Trifoliate Leaf. B. Photo of a measurable trifoliate leaf (left) and one that has yet to develop and thus is not scored as a leaf (right).

e) *Total Number of Leaves and Leaflets:* Count the number of unifoliate leaves (Fig. 5), which are the first two leaves to develop and grow during germination. Typically, they look heart-shaped and there are usually no more than two total per plant. Trifoliate leaves develop later than unifoliate leaves and a single trifoliate leaf consists of a set of three leaflets (Fig. 5); each leaflet of a trifoliate leaf should be counted as an individual leaflet, not leaf. Thus, a single set of three leaflets in a trifoliate leaf should be counted as one leaf (or three separate leaflets).

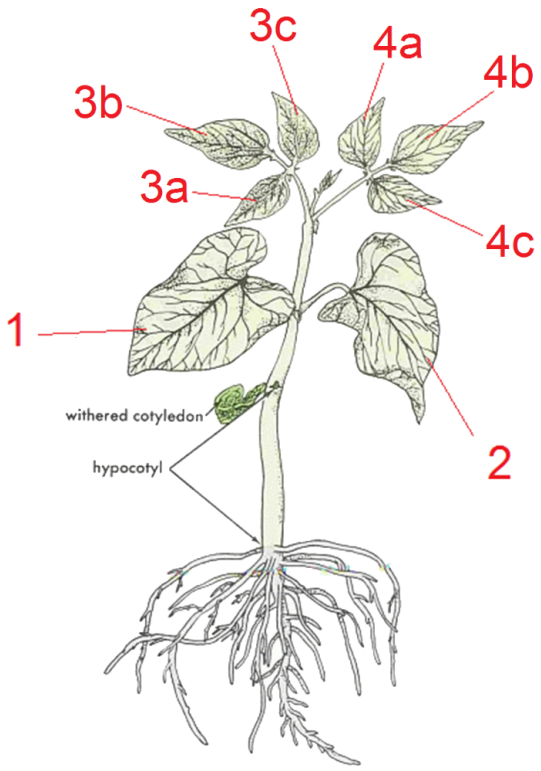


Figure 5. Counting Leaves and Leaflets. Leaves numbered 1 and 2 are unifoliate leaves. Leaflets 3a-c collectively comprise a trifoliate leaf. Leaflets 4a-c collectively comprise a different trifoliate leaf. Image adapted from http://ohhs.ohsd.nt/~brick/pla/images/plai_seeds_dicots_bean.jpg.

f) *Water Chemistry:* One student (or group) must collect data on pH, ammonia, nitrite, and nitrate for the class. Please use an aquarium water chemistry testing kit. Measurements must be taken weekly, but daily measurements are strongly encouraged.

g) *Biomass of Shoot and Root (LAST WEEK ONLY):* These measurements must be done immediately after final trait measurements on the same day of the last week (Week 3). They CANNOT be done sooner because they will permanently damage the plant!
- *Wet weight of shoot*: Obtain a scissor and cut the plant at your permanent marker marking of the main stem trunk diameter (see Main Stem Trunk Diameter). Remove all straw label clippings. Weigh in metric units.
- *Wet weight of root:* Remove all aquaponic medium (clay pebbles, in aquaponic system) or potting soil (in non-aquaponic soil system) from root. Pat dry with paper towel and weigh in metric units.
- *Wet total biomass:* Combine the shoot and root of a plant and weigh in metric units.
- To obtain dry weights, place one plant (shoot and root) into its own brown bag. Label each bag with its identification info (student name, specimen number, date). Keep each bag open, allowing the plant to air dry. Store bags in a dry, warm room. Let them sit for at least one week to allow adequate drying. Afterwards, weigh the shoot and the root of each plant separately. To obtain total biomass, combine the shoot and root of a plant and weigh in metric units. (NOTE: This is a very crude technique to acquire dry weight; many more sophisticated and accurate methods exist!)

h) *Note on Statistical Analyses:* Below are guidelines for statistical analysis.
- To compare categorical variables between treatments (aquaponics vs. soil), a chi-square test can be employed.
- To compare continuous variables, student's t-test can be used if parametric assumptions are satisfied. Otherwise, use Mann-Whitney U-test.

Datasheet

STUDENT NAME: DATE:

AQUAPONIC PLANTS

SPCMN #	STAGE	#C	PH (cm)	HYP (cm)	SD (mm)	#Lf_U	#Lf_T	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )

SOIL PLANTS

SPCMN #	STAGE	#C	PH (cm)	HYP (cm)	SD (mm)	#Lf_U	#Lf_T	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )	Lf_PetL ( )

PH- Plant Height #C- number of cotyledons

HYP - Hypocotyl Length

SD - Main Stem Trunk Diameter (at base; just above root; marked with permanent marker) ALL LENGTH MEASUREMENTS MUST BE IN METRIC UNITS!

#Lf- Number of Leaves (U: unifoliate; T: trifoliate)

Lf_PetL - Leaf Petiole (+ Petiolule) Length (Please denote # of designated leaf)

Materials

The following is a list of items needed per student to accomplish this experiment. Each student should have access to the following:

Item, Qty per student, Qty per 25 students

- 3.5" Netted Pots, 3, 21
- 3.5" Soil Pots, 3, 21
- PLANT!T® Clay Pebbles, varies, 1 bag (10 L)
- Miracle-Gro® Potting Mix, varies, 1 bag (35.3L)
- Permanent Marker, 1, 25
- 6" Ruler (preferably transparent), 1, 25
- 12" Ruler (preferably transparent), 1, 25
- Plastic Drinking Straw (any brand), 1, 25
- Datasheet (see Student Outline), 1, 25
- Red Kidney Beans (GOYA®), 6, 1 bag (454 g)
- Paper Towel, 1, 1 roll

Notes for the Instructor

The student handout provides an overview of the entire set-up and protocols for each week. This section furthers those descriptions for the instructor by including: (i) the items needed to construct the experimental (aquaponic) system and control (non-aquaponic soil) system, (ii) optional measurements, and (iii) extension activities.

1. Items Needed to Construct the Experimental (Aquaponic) System and Control (Non-Aquaponic Soil) System

Below are materials used to construct the aquaponic unit at Nyack College (Also see Supplement "ABLE2015 Aquaponics Park Huster Mata.pptx" in Appendices). For a more thorough overview about designing and constructing aquaponic systems, see Bernstein (2011).

Materials Needed to Construct Aquaponic System (based on system built at Nyack College in January 2013):

- Growing Bed (Fig. 6): Botanicare® Plant Energy Products 14" x 24" x 7" Microgarden Tray (GT14x24)
- Fluorescent Lighting: Hydrofarm® Designer T5 2 foot 4 tube fixture
- Shelving: Wire Shelving Unit (36" x 16" x 36")
- Plumbing: Aquaponic system – 0.5" Tygon tubing, PVC elbows, and one spray bar. Other - Checkvalves may be needed for tubing for aerators or some filters.
- Fittings (Fig. 6): 1" Bulkhead Fittings (threaded on the outside and inside, the latter is for fitting pipescreen or pipescreen extension), Two (2) Botanicare® Pipescreen (matched to bulkheads), Two (2) Botanicare® Pipescreen extensions (matched to pipescreen)
- Netted Pots: 3.5" diameter (21 in total)
- Pump: Marineland (trademark of AQUARIA, Inc.) Mini-Jet® 404 (to be placed within aquarium)
- Aquarium and Aquarium Stand: 40-gallon breeder aquarium (36" x 18" x 16"); glass canopy is optional but strongly recommended to prevent corrosion or rotting of shelving support stand (see below); stand is not necessary (but is aesthetically pleasing)
- Growing Bed Support Stand: Some sort of support stand that surrounds the aquarium and supports the wire shelving unit should be purchased or constructed, see Figs. 7-8. We constructed one out of wooden boards and plywood.
- Automated Fish Feeder: Penn Plax ® Daily Double II Fish Feeder
- Submersible aquarium heater: Any brand should do, but consult a local aquarist for model/wattage appropriate for your aquarium.
- Electric Filter: Any internal or external filter that combines mechanical and chemical filtration is suitable. Consult local aquarist for model appropriate for your aquarium.
- Aquarium Over-Flow Apparatus (as a safety precaution and flood prevention): See http://youtu.be/65yVr7DiDls
- Aquarium Water Chemistry Test Kit: Any brand should be sufficient so long as it includes tests for pH, ammonia, nitrites, and nitrates.
- Timers: Minimum of two is required, one for fluorescent lighting of aquaponic system and another for internal pump.
- Aquarium fish: At Nyack College, the fish species described in pgs. 184-197 in Kingsolver (2006) were acquired.
- Fish food: Tetra® Tetramin® Fish Flakes (set in the automated fish feeder).
- Zip-ties: Multiple applications

Materials Needed for Non-aquaponic (Soil) System (based on system constructed at Nyack College in January 2013):

- Growing Bed: Botanicare® Plant Energy Products 14" x 24" x 7" Microgarden Tray (GT14x24)
- Fluorescent Lighting: Hydrofarm® Designer T5 2 foot 4 tube fixture
- Shelving: Wire Shelving Unit (36" x 16" x 36")
- Standard Square Potting Soil Pots: 3.5" width (21 total needed)
- Timer: Timer is needed for fluorescent lighting of non-aquaponic (soil) system
- Zip-ties: Multiple applications.

Figure 6. Botanicare® Growing Bed and Fittings. A. Aerial view of growing bed with fastened bulkheads, extensions, and pipescreen. Inset shows disassembled extensions and pipescreens. Only the drain has extensions, of which two are used. The aquarium water from pump enters through the source (i.e., top pipescreen), which does not have any extension. B. Side view of growing bed with fastened bulkheads. Inset shows disassembled bulkheads.

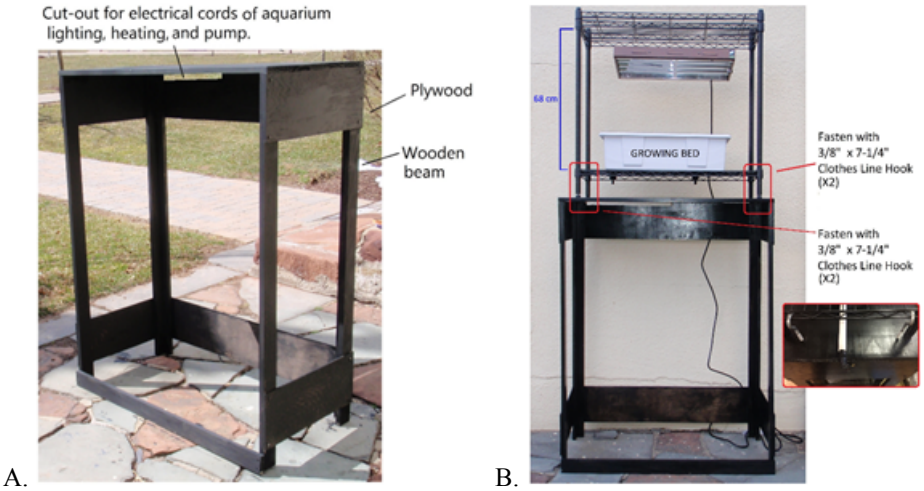


Figure 7. Growing Bed Support Stand. A. Completed support stand, painted. B. Support stand with wire shelving and lighting. Bottom shelf of shelving unit is supported by clothes line hooks (see inset).

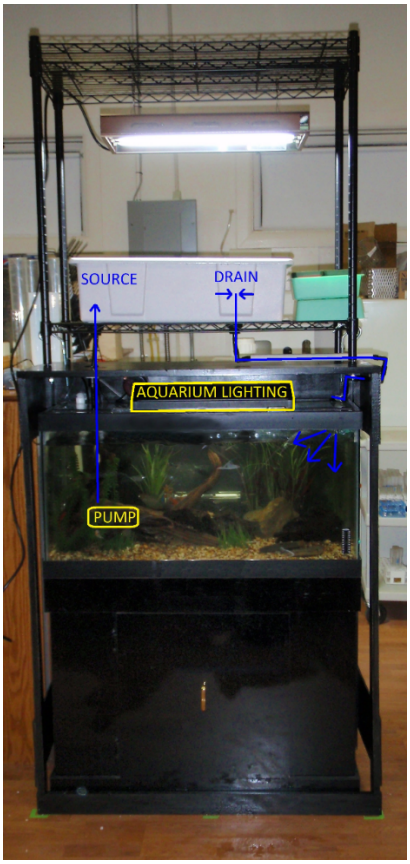


Figure 8. Aquaponic System at Nyack College. Flow of water shown.

Notes Regarding Regular Operation of Aquaponic System and Non-aquaponic (Soil) System:
For fluorescent lighting, each system should be set to a 12-hour light: 12 hour off cycle using a timer.

2. Summary of Measurements, Including Optional Characters.

Optional Plant Measurements:
Below is a list of additional measurements that can be acquired during lab sessions. We have opted to exclude almost all of these from the main study because they were usually too time-consuming, student data collection of these variables was consistently unreliable, and/or the plants had not developed these structures within the regular duration of the study.

a) Epicotyl Length (Type of Variable: Continuous) – Length between cotyledon (or cotyledon scar) and node of unifoliate leaves (junction of unifoliate leaf petioles and main stem).
b) Leaf Area (Type of Variable: Continuous) - Leaf area can be measured in either of two ways:
   i. Length x Width: The length of the leaf (or leaflet) is the distance between the base of the leaf (at its petiole) and the distal point of the leaf. Width would be the widest width that is measured perpendicularly to the length measurement. Compared to method b (below), this method is less accurate but less time-consuming. In essence, you are measuring the "box" within which the leaf would fit.
   ii. Area Outline: A more accurate measurement of leaf (or leaflet) area can be achieved using a laminated sheet of graph paper. Students can flatten a leaf against the graph paper. To achieve maximum flattening, a thin sheet of transparency paper can be laid over the leaf and fastened using a paperclip. Students can then outline the leaf on the transparency against the graph paper or take a quick photograph of the leaf against the graph paper background. Area can be calculated manually from the graph paper grids or using an image-processing software program (e.g., ImageJ).
c) Number of Axial Buds (Type of Variable: Categorical) - Count total number of axial buds.

d) Number of Flowers (Type of Variable: Categorical) - Count total number of flowers, if present.
e) Number of Fruits (Type of Variable: Categorical) - Count the total number of fruits (also called seed pods or bean pods).

Below is a summary of all described characters that can be measured by students on red kidney bean plants (Table 1).

Table 1. Summary of measurements described in present work.

Characters	Measurements	
	Weekly	End of Expt
Main Stem Trunk Diameter marked base of main stem)	X	X
Plant Height (marked base of main stem to base of terminal bud)	X	X
Hypocotyl Length (marked base of stem to cotyledon)	X	X
Number of Unifoliate Leaves	X	X
Number of Trifoliate Leaves (and/or leaflets)	X	X
Unifoliate Leaf PetL = Length of Unifoliate Leaf Petiole (node to base of unifoliate leaf)	X	X
Trifoliate Leaf PetL = Length of Trifoliate Leaf Petiole + Petiolule (main stem node to base of lateral leaflets + base of lateral leaflets to base of terminal leaflet)	X	X
Number of Cotyledons	X	X
Epicotyl Length*	X	X
Leaf Area*	X	X
Number of Axial Buds*	X	X
Number of Flowers*	X	X
Number of Fruits (Seed Pods)*	X	X
Total Biomass (Wet and Dry)		X
Shoot Biomass (Wet and Dry)		X
Root Biomass (Wet and Dry)		X

*Optional

3. Optional Extension Activities.

a) Given the variety of data that will be collected, advanced students could carry out a Principal Components Analysis (PCA) on their dataset.
b) Quality and quantity of protein content of plants between treatments.
c) Discrete foraging niches in fishes (Kingsolver, 2006). In this activity, chi-square tests are used to analyze tally data of fish feeding among different zones ("niches") within an aquarium.
d) Using an ecosphere to understand cellular respiration in terms of matter and energy within ecosystems (White & Maskiewicz, 2014).

A. 

B. 

Figure 9. Aquaponics at Nyack College. A. Aquaponic system with plants (Fall 2013) B. Comparisons of plant growth in aquaponic and non-aquaponic systems (Fall 2014). 3.5" circular netted pots (left) and 3.5" square potting soil pots shown (right).

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Appendix
Sample Results

Results from three separate experiments carried out by Park and Nyack College students are provided as supplementary Microsoft® Excel datasheets:

1. Basic Experiment (Fig. 9B, Filename: "Aquaponics EXPT1 REFERENCE.xls"): Aquaponic plants (n=21) vs. Non-aquaponic plants (n=21). Results from Fall 2014 using methods described above. Regarding fluorescent lighting, both systems received 12 hour light : 12 hour off. Aquaponic plants were grown in PLANT!T® clay pebbles using 3.5" circular netted pots; one plant per pot. Non-aquaponic plants were grown in Miracle-Gro® Potting Mix using 3.5" square potting soil pots; one plant per pot. The aquaponic system's submersible pump was set at a 90 min on : 90 min off alternating cycle. Due to logistical constraints, non-aquaponic plants were watered up to twice every 2-3 days. All other variables were the same between treatments. Fig. 9B shows the growth of red kidney bean plants in our aquaponic system and non-aquaponic (soil) system during this experiment. Axial buds were recorded only in this experiment.
2. Effect of Light Duration (Filename: "Aquaponics EXPT2 Light Duration.xlsx"): Aquaponic plants (n=21) vs. Non-aquaponic plants (n=21). Results from Fall 2014. Regarding fluorescent lighting, aquaponic system received 24 hour light : 0 hour off, but non-aquaponic system received 12 hour light : 12 hour off. Aquaponic plants were grown in PLANT!T® clay pebbles using 3.5" circular netted pots; one plant per pot. Non-aquaponic plants were grown in Miracle-Gro® Potting Mix using 3.5" square potting soil pots; one plant per pot. The aquaponic system's submersible pump was set at a 90 min on : 90 min off alternating cycle. Due to logistical constraints, non-aquaponic plants were watered up to twice every 2-3 days. All other variables were the same between treatments.
3. Effect of Density (Filename: "Aquaponics EXPT3 Density.xlsx"): Aquaponic plants (n=53) vs. non-aquaponic plants (n=20). Results from Fall 2013. Regarding fluorescent lighting, both systems received 12 hour light : 12 hour off. There were more than double the number of aquaponic plants than non-aquaponic plants. Aquaponic plants were grown in PLANT!T® clay pebbles using 2" circular netted pots; one plant per pot. Non-aquaponic plants were grown in Miracle-Gro® Potting Mix using 3.5" square potting soil pots; one plant per pot. The aquaponic system's submersible pump was set at a 90 min on : 90 min off alternating cycle. Due to logistical constraints, non-aquaponic plants were watered up to twice every 2-3 days. All other variables were the same between treatments.