NEED SALT ON THAT MANGROVE?

Introduction into Serial Dilution

Rationale and Objective

This activity uses mangrove seeds and is best done in very late fall or early winter when the mangrove seeds have just been collected. A follow-up activity on hardening of mangrove seedlings is included and will serve to revisit serial dilution.

Teacher Background

Mangroves are an interesting group of plants. Most species have interesting root adaptations that aid in support and in gas exchange. They all exhibit vivapary, which means the seedling begins to develop before dropping from the mother plant (the "germinans" portion of the black mangrove scientific name refers to this trait). This seems to give them a "head start" once they implant, increasing chances of survival. In addition, the fruit can float-- aiding in their dispersal.

Unlike most other woody plants, mangroves grow in salty water. An advantage of growing in such an environment may be lack of competition. However, salinity acts as a limiting factor for mangroves, as well. Each mangrove species has particular salinity tolerance levels and requirements at which it grows best. Black mangroves tend to live in brackish environments.

For more information, refer to the Coastal Roots **"Learning about Black Mangroves"** and **"Germination of Black Mangrove Seeds"** Fact Sheets.

Louisiana Science Benchmarks

LOUISIANA BENCHMARKS:

SI-H-A1, A2, A3, A7	LS-H-C6, D3, F2, F3	SE-H-A3, C1
SI-M- A1, A2, A3, A5, A7, A8	LS-M-A3, C4	SE-M-A2

Procedure: Germinating Mangrove Seeds

1. Review germination stages of black mangrove seeds.
   Once placed in water, the black mangrove seed will float and the seed coat will loosen and fall off. Once the seed coat is shed, the cotyledons swell and open and the primary root will emerge from the seed's widest end. The speed at which this occurs depends upon water temperature and salinity. In low salinity and high salinity, the seed coat falls off slowly.
   In brackish water the seed coat is shed quickly, allowing the seedling to implant in its favored habitat. Higher temperatures also seem to increase the rate of development. (Black mangrove seedlings can stay alive in the water for only a few days. It is important that you be prepared to plant as soon as the cotyledons and primary root appear.)
2. After reviewing this information with the students, distribute materials for dilution lab:

Per individual	Per group
Serial dilution worksheet	Food coloring-(The little bottles available in grocery stores work well and are economical. Red, green and blue work best.)
Pen or pencil	Medicine dropper (pipette)
	One Chemplate or 12 small transparent plastic cups. Chemplates are available from chemical and environmental supply companies. Small, transparent plastic cups also work well.
	Wastewater container (plastic cup or beaker)
	Tap water
	Paper towels (to clean up spills)

Conduct the serial dilution exercise (Intro. to Serial Dilution - Student Worksheet #1).

NOTE: Concentrations of solution components are frequently expressed in terms of relative concentrations. These concentrations are stated as fractions of the whole. For example, adding 3 ml chocolate syrup to 97 ml milk would create a 100 ml solution. However, regardless of the quantity of chocolate milk we wish to produce or the unit of measure we use, we can obtain chocolate milk of this same concentration by adding 3 **parts** (or units) of syrup per every 100 parts of solution we wish to create. This relationship is referred to as " parts per hundred" or percent. However, in reporting concentrations in science, the solute quantities are frequently quite small and concentrations are frequently expressed in parts per thousand (ppt), parts per million (ppm), or even parts per billion (ppb). Serial dilution activities help us to "visualize" these concentrations.

3. Teach students how to use the salinity **refractometer**. Have students use the salinity refractometer to check the accuracy of their dilutions. Information on how this instrument is used is available online at the Coastal Roots website.

4. Distribute a copy of the COASTAL ROOTS EXPERIMENTAL DESIGN DIAGRAM to each student. This diagram is included in the Coastal Roots Experimental Design lesson. Working as a class, have the students design an investigation to determine the salinity at which mangroves will germinate "best". Be sure to have them operationally define "best"!

   As this activity is student-designed, hypotheses and dependent variables will vary with the class. Materials and quantities of each will also vary but are likely to include:

   Aquarium Salts (purchased from pet store or biological supply facilities)
   Balance or scale to measure salts
   Refractometer
   Measuring cups or beakers
   Water (Do not use Distilled water.)
   Plastic buckets (large enough to place enough water to cover seeds)
   Black Mangrove seeds

   IMPORTANT POINTS TO NOTE:

   A. Limiting salinity to less than 25 ppt will decrease death of seedlings.
   B. Having the students produce the several salt solution concentrations will provide opportunity for review of metric and measurement skills as well as practicing their new serial dilution and refractometer-use skills.
   C. Seeds soaked in tap water took about 1 week to emerge.
   D. Planting instructions for black mangrove seeds is included in **Planting Your Coastal Roots Seeds** information sheet

Procedure: Hardening of Mangrove Seedlings

As the time to plant the seedlings in a restoration area draws near, black mangroves should be "hardened". This means they should be exposed to conditions like those in which they will be growing; in other words, they should be grown in a brackish or saltwater solution. But how salty should the water in which they "harden" best? Again, an opportunity for student-designed inquiry!

1. Distribute a copy of the COASTAL ROOTS EXPERIMENTAL DESIGN DIAGRAM to each student. Working as a class or in small cooperative lab groups, have students design an investigation to create an environment most like the one to which they are being transferred. Students should be provided with as much information about the restoration area as possible. Once again, the hypotheses and dependent variables may slightly vary but the necessary materials will include:
    - Seedlings
    - Salt solutions
    - Measuring tools (rulers, beakers, balance or scale)
    - Watering cans
    - Refractometer
    - Separate pools (troughs) for each level of the independent variable (each salt concentration)

NOTE---Limiting salinity concentrations to less than 25 ppt is suggested.

Water levels in the troughs should be monitored daily and should remain within a few centimeters of the top of the pots in which each tree is planted. Mangrove seedlings will die quickly if allowed to dry out. It is critical that water levels in the pools be checked daily and that the refractometer is used to check and adjust the salinity of the pools.

References

Wetlands and Rain Forests
http://www.epa.qld.gov.au/environment/school/wetlands/
(Click on: Mangroves)

On-Line Resources

MANGROVES

Florida Forest Trees: Black Mangrove http://aris.sfrc.ufl.edu/4h/Ecosystems/Mangrove/mangrove.html

Wetlands and Rain Forests www.epa.qld.gov.au/environment/school/wetlands/
(Click on: Mangroves)

SERIAL DILUTION

The Egret's Watch, Spring 1994 www.leeric.lsu.edu/outreach/egrets94.htm

Need A Little Salt On That Mangrove?

Student Name: ___________________

Introduction Into Serial Dilution

Mangroves are an interesting group of plants. Most species have interesting root adaptations that aid in support and in gas exchange. They all exhibit vivapary, which means the seedling begins to develop before dropping from the mother plant (the "germinans" portion of the black mangrove scientific name refers to this trait). This seems to give them a "head start" once they implant, increasing chances of survival. In addition, the fruit can float-- aiding in their dispersal.

Unlike most other woody plants, mangroves grow in salty water. An advantage of growing in such an environment may be lack of competition. However, salinity acts as a limiting factor for mangroves, as well. Each mangrove species has particular salinity tolerance levels and requirements at which it grows best. Black mangroves tend to live in brackish environments.

Some species of mangrove have been raised in pots where they have grown successfully and flowered when given only fresh water. Experimentation, however, has shown that the best mangrove growth occurs when the plants live in seawater diluted with fresh water.[1] What salt levels will our black mangroves germinate best? Should we maintain a brackish environment in our nurseries? How can we go about measuring salinity levels?

Before beginning to experiment with black mangrove seeds, let's consider a little mangrove seed biology and learn a few measurement techniques.

Once placed in water, the black mangrove seed will float and the seed coat will loosen and fall off. Once the seed coat is shed, the cotyledons swell and open and the primary root will emerge from the seed's widest end. The speed at which this occurs depends upon water temperature and salinity. In low salinity and high salinity, the seed coat falls off slowly. In brackish water the seed coat is shed quickly, allowing the seedling to implant in its favored habitat. Higher temperatures also seem to increase the rate of development. (Black mangrove seedlings can stay alive in the water for only a few days. It is important that you be prepared to plant as soon as the cotyledons and primary root are evident!)

Your seedlings should be "hardened" before planting them in a restoration area. This means they should be exposed to conditions like those in which they will be growing, in other words, they should be grown in a brackish or saltwater solution. But how salty should the water in which they germinate or in which they "harden" best and how can this salt concentration be measured and regulated? The answer begins with serial dilution.

Serial dilution refers to the addition of specific quantities of water (or other solvent) to a solution of known strength---in other words, diluting it by controlled amounts. To determine the "best " concentration to harden the mangrove seeds, you'll need to have a little practice with serial dilution. Once you obtain a copy of the COASTAL ROOTS SERIAL DILUTION WORKSHEET, you'll be ready to begin.

Once you've completed the serial dilution exercise, you will learn how to use a salinity refractometer. The refractometer will be used to measure salinity of the water used to harden your mangrove trees.

Once you've mastered refractometer use, you'll be ready to start investigating the salinities at which the trees harden best. Your teacher may set maximum salinity levels--- (While you are encouraged to experiment, remember we don't want to kill trees!)

Wetlands and Rain Forests *www.epa.qld.gov.au/environment/school/wetlands*[1]

Coastal Roots · Sea Grant Louisiana · Barataria-Terrebonne National Estuary Program · Coalition to Restore Coastal Louisiana · LSU AgCenter Research & Extension

Need A Little Salt On That Mangrove? Introduction Into Serial Dilution

Student Name: ___________________

Concentrations of solution components are frequently expressed in terms of relative concentrations. These concentrations are stated as fractions of the whole. For example, adding 3 ml chocolate syrup to 97 ml milk would create a 100 ml solution. However, regardless of the quantity of chocolate milk we wish to produce or the unit of measure we use, we can obtain chocolate milk of this same concentration by adding 3 **parts** (or units) of syrup per every 100 parts of solution we wish to create. This relationship is referred to as " parts per hundred" or percent. However, in reporting concentrations in science, the solute quantities are frequently quite small and concentrations are frequently expressed in parts per thousand (ppt), parts per million (ppm), or even parts per billion (ppb). Serial dilution activities help us to "visualize" these concentrations.

MATERIALS:

Per individual	Per group
Serial dilution worksheet	food coloring
Pen or pencil	medicine dropper (pipette)
	One Chemplate or 12 small transparent plastic cups
	Wastewater container (plastic cup or beaker)
	Tap water
	Paper towels (to clean up spills)

PROCEDURE:

Working in your cooperative groups, have your materials manager obtain the necessary materials. Read the procedures. If the instructions seem vague, ask for clarification before proceeding.

→ Note: If you are using a Chemplate, the little cups are numbered. Locate cup #1.
If you are using plastic cups, each needs to be numbered before you begin.

1. Place 10 drops of food coloring into cup 1. The concentration of this food coloring is 10%.
2. Use your dropper to move 1 drop of solution from cup 1. Place it in cup 2. (Return any extra to cup 1). Rinse the dropper in the wastewater container, and then add 9 drops of tap water to cup 2. What is the concentration of the solution in cup 2? __________________
3. Remove 1 drop of solution from cup 2. Place it in cup 3. Rinse the dropper and then add 9 drops of water to cup 3. What is the concentration of the solution in cup 3? _______________
4. Repeat step 3 for each of the remaining cups (ending with 12).
5. Complete the lab problems below.
6. Clean up and return materials.

LAB PROBLEMS:

1. In which cup is the food coloring concentration: 1 ppt____ 1 ppm____ 1 ppb_____ ?

2. What is the number of the first cup in which you can not see color?______

3. What is the concentration of the solution in this cup? Cup #_____ Concentration____

4. Is there any food coloring in that cup? Explain your answer.

5. Describe how to make a 4 ppt salt solution.

Need A Little Salt On That Mangrove? Introduction Into Serial Dilution

Student Name: TEACHER's KEY

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MATERIALS:

Per individual
- Serial dilution worksheet
- Pen or pencil

Per group
- food coloring
- medicine dropper (pipette)
- One Chemplate or 12 small transparent plastic cups
- Wastewater container (plastic cup or beaker)
- Tap water
- Paper towels (to clean up spills)

PROCEDURE:

Working in your cooperative groups, have your materials manager obtain the necessary materials. Read the procedures. If the instructions seem vague, ask for clarification before proceeding.

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1. Place 10 drops of food coloring into cup 1. The concentration of this food coloring is 10%.
2. Use your dropper to move 1 drop of solution from cup 1. Place it in cup 2. (Return any extra to cup 1). Rinse the dropper in the wastewater container, and then add 9 drops of tap water to cup 2. What is the concentration of the solution in cup 2? 1 part per hundred (or 1 %)
3. Remove 1 drop of solution from cup 2. Place it in cup 3. Rinse the dropper and then add 9 drops of water to cup 3. What is the concentration of the solution in cup 3? 1 part per 1000 (1 ppt)
4. Repeat step 3 for each of the remaining cups (ending with 12).
5. Complete the lab problems below.
6. Clean up and return materials.

LAB PROBLEMS:

1. In which cup is the food coloring concentration: 1 ppt__3__ 1 ppm__6__ 1 ppb__9__ ?

2. What is the number of the first cup in which you can not see color? Answers may vary but generally, cup 6 or 7.

3. What is the concentration of the solution in this cup? Cup #_____ Concentration Will depend upon answer to problem 2.

4. Is there any food coloring in that cup? Explain your answer.
Yes but it is in such low concentration, it cannot be seen.

5. Describe how to make a 4 ppt salt solution.
Add 4 g salt to 996 ml of water. (Or 0.4 g salt to 99.6 ml water)