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Information				
Introduction/Abstract to	This lesson plan uses real-world ecological dynamics to introduce students to the			
Lesson Plan	scientific method. The students will learn the process of observation, hypothesis,			
	experimental design, data collection, and drawing conclusions that support or do not			
	support their hypotheses via a simple, classroom experiment.			
Learning Objectives	At the completion of this lesson, students will be able to describe the scientific			
	method, formulate hypotheses, setup an experiment, collect data, and plot data on a			
	graph, evaluate and interpret results, and make conclusions about ecological dynamics			
	that amphibians experience in their wetiand habitats.			
Appropriate Grade Levels	9-12			
Group Size/# of students	<30			
activities are designed for				
Setting (e.g. indoors,	Indoor classroom. Egg masses will be collected by the teacher outdoors.			
outdoors, lab, etc.)				
Approximate Time of	~45 minutes for the lesson. It will take approximately 1.5 -3 months for tadpoles to			
Lesson	metamorphose with periodic 20-30 minute teaching points.			
Resources Needed for	Internet access, computer/notebook for taking notes			
Students				
Resources Needed for	4 Clear Tupperware containers or 4 small aquariums. Frozen spinach (or fish flakes)			
Educators	Dechlorinated tap water or pond water, small aquarium net. Salt, teaspoon, Supplied			
	datasheets for data recording (Found on website). (Optional: scale for weight			
	measurements, water dechlorinator).			
Apps/Websites Needed	Access to YouTube			
Lesson Activity	1. Introduce students to the project using PowerPoint (on website). Information			
	on habitat, characteristics, requirements, and life cycle will be introduced.			
	Additionally, the scientific method will be outlined and the experiment's			
	protocols will be introduced and discussed.			
	2. Have students gather around the egg mass that you collected and engage the			
	class in a discussion based on what they just learned.			
	 Set up the experiment with student observation (Protocols in PowerPoint). Students should write bunchases and justify them 			
	4. Students should write hypotheses and justify them.			
	5. Over the next new weeks, students observe the developments and record then			
	6 Students wait for metamorphosis and date and number that emerge each day			
	(generally tadnoles metamorphiss and date and number that energy each day			
	first) The response variable that we are interested in is the amount of time to			
	metamorphosis.			
	7. Optional: Have one or two students weigh the metamorphs as they emerge			
	and record the weight of each. This will vield an additional response variable of			
	"weight at metamorphosis". This can be tricky though, as the froglets are very			
	small, difficult to handle, and can be surprisingly jumpy.			
	8. Place the metamorphs in a separate, container to be released to a wetland			

	 (husbandry techniques outlined below). 9. Enter data into Excel and create a graph using the time to metamorphosis and/or weight at metamorphosis according to treatment. 10. Questions for students: Is there a pattern in the data? What is the pattern? 11. Have students write lab report on the activity. A lab report format sheet and 				
	suggested rubric are available on the website.				
Final Product	A lab report on the experiment including an introduction (including independent research accomplished by the student), hypotheses, a description of the experimental design, a graph depicting the results (can be excel or hand drawn), and the conclusions with clear assessment of whether or not their original hypothesis was supported.				
	plementary materials.				
NC Essential Standards	 Bio.1.2.1: Explain how homeostasis is maintained in the cell and within an organism in various environments (including temperature and pH). To maintain homeostasis, tadpoles must maintain a water balance. If tadpoles are placed in a habitat with high solute (hyperosmotic) concentrations, they will need to use energy to actively pump out excess salts to stay alive. This means that energy that would otherwise be used for growth is used for the homeostatic demands. How will that affect their weight at metamorphosis and how long that it takes to metamorph? (take longer, as they are using up the energy to pump out salts that they would otherwise use to grow) Bio.2.1.4: Explain why ecosystems can be relatively stable over hundreds or thousands of years, even though populations may fluctuate (emphasizing availability of food, availability of shelter, number of predators and disease). Animals face environmental stressors ranging from disease, predators, competition, and food shortages. In each case, animals will adapt or the strongest survive. If only the strongest survive, they will be better able to capitalize on the opportunities by better avoiding predation, exploiting food sources, outcompeting others for resources, and avoiding infection. What can we infer about a tadpole's ability to endure salt stress? Will they be better ow sore off after enduring the osmotic stress? (Answer: depends – they will probably be smaller which may mean fewer breeding opportunities. But, they may be better oble to survive salt vocation and introduction of nonnative species) may impact the environment. We are learning how a "natural" component of ecosystems can affect tadpoles but many ecosystems are laced with various pollutants and chemicals. Discuss: What about human caused pollutants- how might the principles we learned in this lesson help us understand how tadpoles are affected by pollutants and other introduced chemicals? (Can be lethal, or they can just aff				

	tissues to help get rid of excess salts.				
	Bio.3.4.2+Bio.3.4.3: Explain how natural selection influences the changes in species				
	over time, and how various agents can influence natural selection.				
	Questions for discussion: How might salt stress influence the natural selection				
	of the tadpoles in that salt-invaded habitat?				
	• Answer: To adapt to this environment, tadpoles must be able to excrete the excess salt. Some individuals may have an adaptation that allows them to excrete the salts with very little energetic cost. Therefore they may have more				
	energy to acquire food, grow, escape predators, and metamorphose.				
	Therefore, these individuals may have higher rates of reproduction and				
	therefore pass the advantageous adaptation to its offspring (assuming it is a				
	heritable trait).				
	Bio.4.1.2+Bio.4.2.2: Summarize the relationship among DNA. proteins and amino				
	acids in carrying out the work of cells and how this is similar in all organisms, Explain				
	ways that organisms use released energy for maintaining homeostasis (active				
	transport).				
	• A portion of the DNA code is translated and transcribed into the amino acids				
	that make up the sodium-potassium pumps (proteins). These active transport				
	pumps are crucial to maintain a proper osmotic balance in all living organisms,				
	including humans. They do require the use of energy that is released from ATP				
	(Adenosine TriPhosphate).				
Next Generation	HS-LS1-3: Plan and conduct an investigation to provide evidence that feedback				
Standards	mechanisms maintain homeostasis.				
	Maintaining osmotic balance is a fundamental process in maintaining				
	homeostasis. This experiment highlights osmotic stress and the costs				
	associated with actively maintaining water balance.				
	HS-LS4-2: Construct an explanation based on evidence that the process of				
	evolution primarily results from four factors: (1) the potential for a spe				
	increase in number, (2) the heritable genetic variation of individuals in a				
	species due to mutation and sexual reproduction, (3) competition for limited				
	resources, and (4) the proliferation of those organisms that are better able to				
	survive and reproduce in the environment.				
	• This experiment may demonstrate that some individuals are better able to				
	tolerate the osmotic stress of a salty larval environment. If these differences				
	are genetic and heritable, the individuals may produce the next generation of				
	tadpoles that can handle osmotic stress better than others and capitalize on				
	resources.				
	• In their lab reports, students should indicate a basic understanding of these				
	principals but also understand that natural selection involves a tradeoff				
	between energetic demands (osmotic balance vs. growth). The particular				
	environment will dictate which particular strategy is more adaptive (salty				
	environments will likely select for ability to regulate osmotic balance well,				
	while other environments may select for the ability to grow fast and				
	metamorphose quickly to escape predation or a food-starved larval habitat.)				

Egg mass pictures:



Sample aquaria:



Experimental Protocols:

After eggs have hatched, place half of the tadpoles into one tank with freshwater. Place the other half into water with added salt (table salt should be fine). Keep in mind, too much salt is lethal. Aim for ~3-4ppt at most (3g of salt/1 Liter of water). Record the day that tadpoles were placed in water (to determine how long it takes for frogs to metamorphose). Care and feed both tanks as equal as possible. See instructions below for raising tadpoles. Once frogs begin emerging from tanks, record the date of emergence, and weigh/measure the tadpoles, and mark whether they were in salt or freshwater treatments. After all/most frogs have metamorphosed, students can enter data into excel, plot the data, and draw conclusions.

Key Questions for Students to answer:

Did salt affect the amount of time that frogs took to reach metamorphosis?

Did salt affect how big the tadpoles were once they reached metamorphosis?

How to collect frog eggs:

Around March, frogs will emerge from hibernation and begin to breed. Luckily, we live in North Carolina with plenty of wetlands (and therefore lots of frogs!). Finding eggs and tadpoles is fairly quick, as frogs are eager to breed and will lay eggs in water-filled roadside ditches, tire ruts, ponds, and swamps. The best time to go looking for eggs is the morning after a big spring rain. Look for gelatinous clumps with black dots in the center (see pictures above). Egg masses float on the surface or may be attached to branches/plants near the surface. To collect the eggs, dip your container below the egg mass and scoop it up with water. Try not to shake or disrupt the egg mass too much. Eggs should hatch about 7 days later, but you'll be able to see them developing inside the eggs. (Note: if they do not show development within a week, you may have unluckily grabbed an unfertilized clutch...)

Tadpole care: Tadpoles are very easy to maintain which makes this an ideal classroom "pet" and activity. Tadpoles feel very vulnerable out in the open and would love some hiding places in their tanks. A few rocks/leaves from outside will be excellent. Since they are cold-blooded, placing them in a sunny window location will help keep them warm. After hatching (~2-7 days) they will feed for a couple days on stored yolk (during this stage they will be immobile and look like they are dead) and will begin foraging after begin to swim freely. Depending on the species that you collect, they may take between 1.5 months to 3 months to fully develop.

They can eat frozen boiled spinach (allow to thaw first), fish flakes, or Spirulina. However, be careful not to overfeed as the excess food will rot and will kill the tadpoles. A filter helps keep water clean – but you do not need a filter if you do a quick water change every week or so. (Note – when tadpoles are small, they are not strong enough to swim against the suction of a filter. Be sure to install a piece of thin sponge, screen, or fabric onto the intake to ensure tadpoles do not get sucked into the filter.) To complete a water change, net the tadpoles into a different water container, rinse the aquarium, and pour the tadpoles back into the original aquarium. You can either use more pond water, or use de-chlorinated tap water (Note: do not use water straight from the tap as the chlorine is lethal to tadpoles). Froglets will sprout back legs first and will remain bipedal in the water for several weeks until the front limbs emerge. They will then climb out of the water onto the sides of the tank or onto a platform and begin to reabsorb their tails in the final stages of metamorphosis. To assist with this process, provide with a platform to sit upon. Good platforms are pieces of floating Styrofoam, or rocks that rise out of the water. It would be ideal to pull these individuals out after they emerge and place them in a separate container lined with a moist paper towel. These frogs are now ready to be released back to the wild, and should be kept in a moist container until you return them to the wetland. As long as you release them within a week, you do not need to feed these individuals, as they do not start feeding for about 7 days. However, if you keep them for longer, fruit flies are an ideal temporary diet.

Notes:

- 1. There is a chance that the egg mass you collect will not develop as fertilization is external and not always successful.
- 2. Be careful about allowing students to touch the tadpoles, as they are very small and fragile. Frog skin easily tears and limbs are easily broken.
- 3. If the water becomes dirty or you decide to change it if you use tap water, be sure to dechlorinate it first (dechlorinators are available at any pet store). Untreated pond/lake/wetland water can be also used to replace/supplement the water in the aquarium.

Appendices: Suggested Lab Report Grading Rubric: 25 possible points (suggested)

Points	Introduction	Experimental	Results	Conclusion/Discussion	Grammar and
		Design			Spelling
5	Well researched, excellent comprehension of material, hypothesis clearly stated	Students relate design to specific question. Protocols outlined clearly. Students show understanding of experimental concepts like standardization, replication, etc.	Students have labeled, legible graph of correct data with figure caption that explains graph and data.	Students demonstrate comprehension by linking results back to original hypothesis (i.e. Original hypothesis was supported or not). And students discuss natural selection implications of osmotic balance using results.	<3 errors
4	Decent background, moderate understanding, hypothesis clearly stated	Student details experimental design but do not link to hypotheses. Protocols outlined. Some understanding of experimental concepts.	Neat and clean graph with correct data. Graph has figure captions but no axis labels.	Students link results to hypothesis but do not clearly state whether they hypothesis was supported. Some discussion of implications towards natural selection.	<6 errors
3	Some background, little understanding, hypothesis mentioned	Students poorly communicate experimental design, protocols barely mentioned, little understanding of experimental concepts.	Graph does not have proper data or mixed up axes. Graph is missing labels or figure caption as well.	Students do not link results to hypothesis, merely restate methods and results. Little or no discussion of broader concepts.	<9 errors
2	Little background information, little comprehension of the topic, no hypothesis	Experimental design is protocols only with no indication that students understand reasoning behind the design or hypothesis.	Graph is incorrect data, lacks axis labels, figure captions, etc.	Students repeat previous sections with zero discussion of implications or relationships.	<12 errors
1	No background research, very little comprehension, no mention of hypotheses	Experimental design is a picture or diagram with little or no text explaining protocols. Hypothesis not mentioned.	Graph is illegible or absent.	Students demonstrate little understanding, do not link the sections together, do not make any broader inferences on work.	>13 errors