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DATA Nugget

Why so blue? The determinants of color pattern in killifish
Featured scientist: Becky Fuller from The University of Illinois

Research Background:

In nature, animals can be found in a dazzling display of different colors and patterns. Color patterns serve as signals to members of the animal's own species, or to other species. They can be used to attract mates, camouflage with the environment, or warn predators to stay away. When looking at the diversity of colors found in nature, you may wonder, why do animals have the color patterns they do? One way to study this question is to look at a single species that has individuals of different colors. This variation can be used to uncover the mechanisms that determine color.

The color polymorphism in bluefin killifish – males display anal fins in blue, red, or yellow. Photos by Tony Terceira.

The bluefin killifish is a freshwater species that is found mostly in Florida. They are found in two main habitats, springs and swamps. An intriguing aspect of this species is that male bluefin killifish are brightly colored with many different color patterns. The brightest part of the fish is the anal fin, which is found on the bottom of the fish by the tail. Some males have red anal fins, some have yellow anal fins, and others have blue anal fins. This variation in color is called a **polymorphism**, meaning that in a species there are multiple forms of a single trait. In a single spring or swamp you may see all three colors!


Becky in the field, with her colleague Katy, collecting fish in the Wakulla Spring.

Becky is a biologist studying bluefin killifish. One day, while out snorkeling for her research, she noticed an interesting pattern. She observed that there were differences in the polymorphism depending on whether she was in a spring or a swamp. Springs have crystal clear water that can appear blue-tinted. Becky noticed that most of the males in springs had either red or yellow anal fins. Swamps have brown water, the color

Name____________________

Becky's family helping her out in the field!

of iced tea, due to the dissolved organic materials in the water. Becky noticed that most of the males in swamps had blue anal fins. After noticing this pattern she wanted to find out why this variation in color existed. Becky came up with two possible explanations. She thought males in swamps might be more likely to be blue (1) because of the **genes** they inherit from their parents, or (2) because individual color is responding to environmental conditions. This second case, where the expression of a trait is directly influenced by the environment that an individual experiences, is known as **phenotypic plasticity**.

Becky had to design an experiment that could tease apart whether genes, plasticity, or both were responsible for male anal fin color. She did this by collecting male and female fish from the two habitat types, breeding them, and raising their offspring in clear or brown water. If a father's genes are responsible for anal fin color in their sons, then fathers from swamps would be more likely to leave behind blue sons. If environmental conditions determine the color of sons, then sons raised in brown water will be blue, regardless of the population origin of their father.

Becky and her colleagues collected fish from two populations in the wild - Wakulla Spring, and 26 Mile Bend Swamp - and brought them into the lab. These two populations represent the genetic stocks for the experiment. Fish from Wakulla are more closely related to each other than they are to fish from 26 Mile Bend. In the lab, they spawned female fish with male fish from the same population: females from Wakulla spawned with males from Wakulla, and females from 26 Mile Bend spawned with males from 26 Mile Bend. After the offspring hatched from their eggs, half were put into tanks with clear water (which mimics spring conditions) and half in tanks with brown water (which mimics swamp conditions). For the brown water treatment, Becky colored the water using 'Instant, De-caffeinated, No-Sugar, No-Lemon' tea. They raised the fish to adulthood (3-6 months) so they could determine their sex and the color of the son's anal fins. Becky then counted the total number of male offspring, and the number of male offspring that had blue anal fins. She used these numbers to calculate the proportion of sons that had blue anal fins in each treatment.

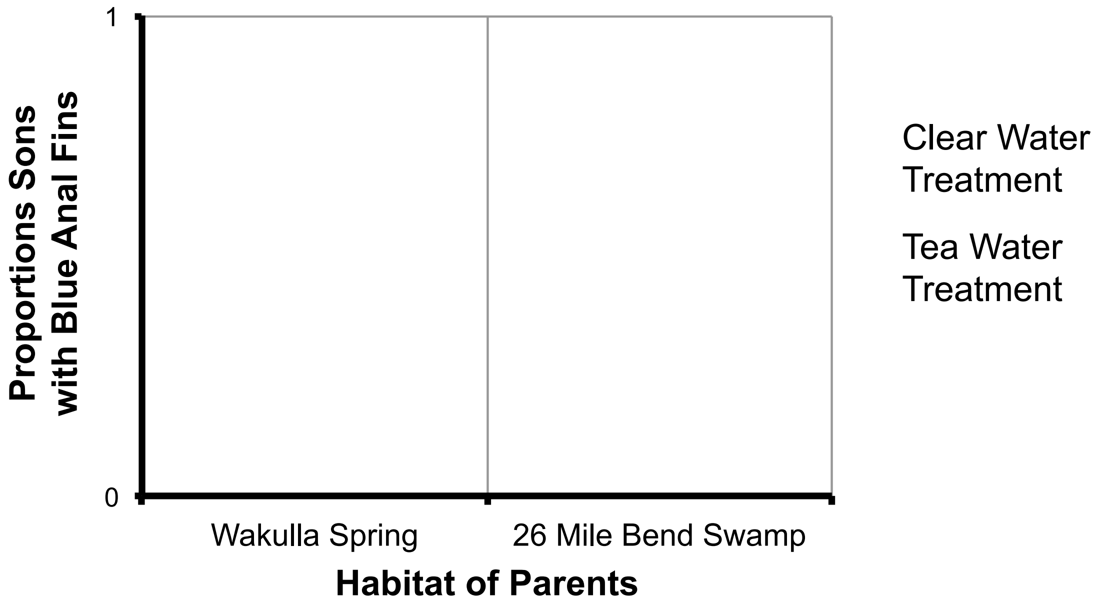
Scientific Question: Is male anal fin color determined by genetics, environment, or both?

What is the hypothesis? Find the two hypotheses in the Research Background and underline them. A hypothesis is a proposed explanation for an observation, which can then be tested with experimentation or other types of studies. Having two alternative hypotheses means that more than one mechanism may explain a given observation. Experimentation can determine if one, both, or neither hypotheses are supported.

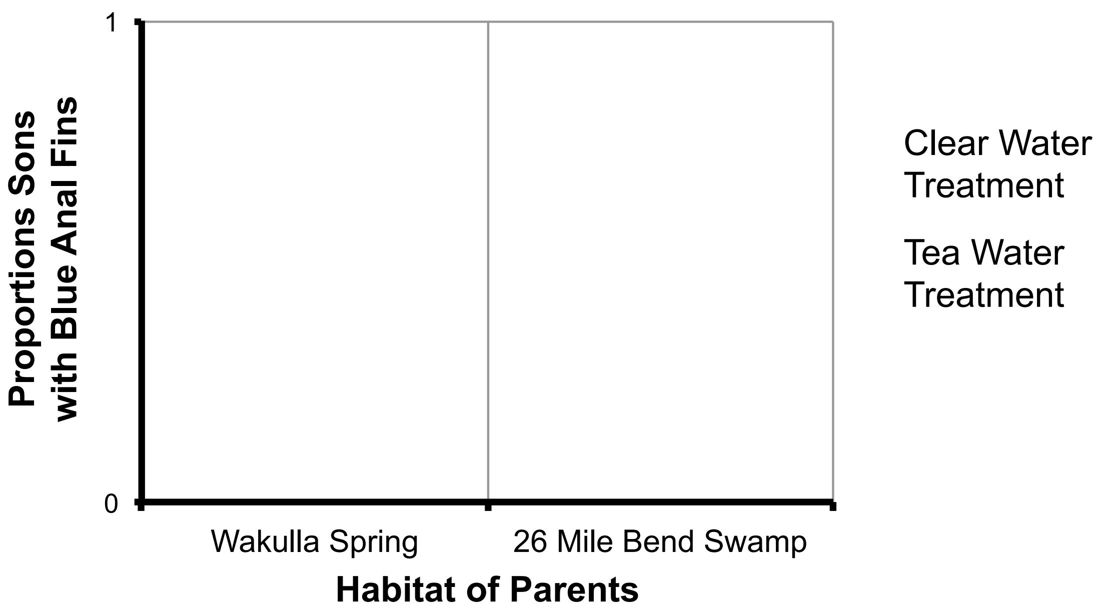
Name____________________

Draw your predictions: Becky hypothesized that the color of the bluefin killifish's anal fin is determined by potentially two factors: (1) the genes they inherit from their parents, and (2) the environment that they are raised in. Below, draw your predictions that arise from each of these hypotheses. In your first graph, draw your predictions if male offspring anal fin color is genetic and determined only by the genes they inherit from their parents. In your second graph, draw your predictions if male offspring anal fin color is plastic and determined only by the environment they are in.

Genetic Predictions



Environmental Predictions



Name____________________

Scientific Data:

Use the data below to answer the scientific question:

Father #	Parent Population	Water Treatment	Total # of Sons	Total # of Sons with Blue Anal Fins	Proportion Sons w/ Blue Anal Fins
1	Wakulla	clear	10	0	
2	Wakulla	clear	33	1	
3	Wakulla	clear	32	0	
4	Wakulla	clear	26	0	
5	Wakulla	clear	15	0	
6	Wakulla	clear	25	0	
7	Wakulla	clear	28	0	
8	Wakulla	clear	17	0	
9	Wakulla	clear	11	0	
1	Wakulla	brown	16	0	
2	Wakulla	brown	23	0	
3	Wakulla	brown	26	0	
4	Wakulla	brown	31	2	
5	Wakulla	brown	31	1	
6	Wakulla	brown	23	1	
7	Wakulla	brown	42	0	
8	Wakulla	brown	50	1	
9	Wakulla	brown	14	0	
10	26 Mile Bend	clear	29	1	
11	26 Mile Bend	clear	21	0	
12	26 Mile Bend	clear	17	1	
13	26 Mile Bend	clear	16	0	
14	26 Mile Bend	clear	31	1	
15	26 Mile Bend	clear	34	6	
16	26 Mile Bend	clear	14	0	
17	26 Mile Bend	clear	31	0	
10	26 Mile Bend	brown	38	11	
11	26 Mile Bend	brown	6	3	
12	26 Mile Bend	brown	24	2	
13	26 Mile Bend	brown	17	3	
14	26 Mile Bend	brown	20	5	
15	26 Mile Bend	brown	28	5	
16	26 Mile Bend	brown	25	0	
17	26 Mile Bend	brown	42	3	

Name____________________

Parent Population	Water Treatment	Average Proportion Sons w/ Blue Anal Fins	Standard Deviation (SD)	Sample Size (N)	Standard Error (SE)
Wakulla	clear		0.010	9	0.003
Wakulla	brown		0.024	9	0.008
26 Mile Bend	clear		0.060	8	0.021
26 Mile Bend	brown		0.156	8	0.055

**Standard deviation (SD) tells us about the amount of variation in the data. A large SD means there is a lot of variation around the mean, while a small SD means the data points all fall very close to the mean. Standard error (SE) is SD divided by the square root of the sample size (N), and tells us how confident we are in our estimate of the mean. A large SE means we are not very confident, while a small SE means we are more confident.*

What data will you graph to answer the question?

Independent variables: ____

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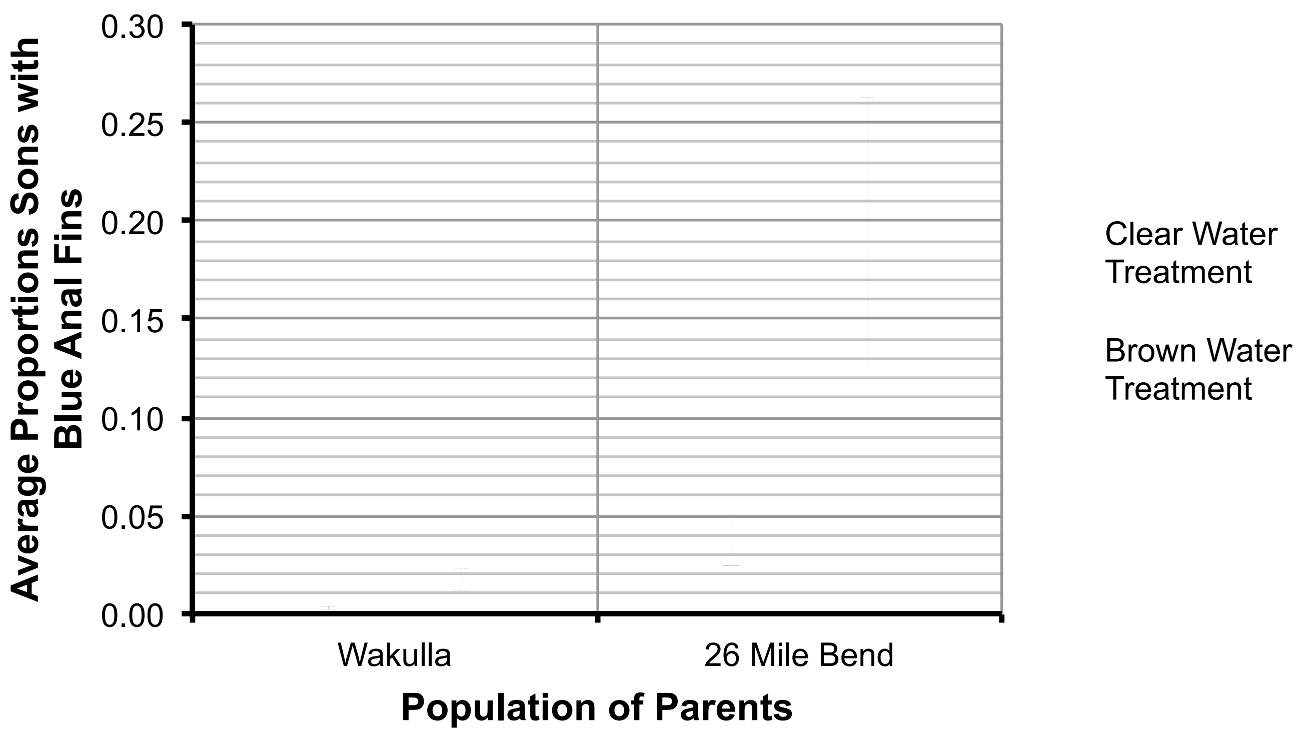
Dependent variable: ____

Draw your graph below: Identify any changes, trends, or differences you see in your graph. Draw arrows pointing out what you see, and write one sentence describing what you see next to each arrow.



Name____________________

Interpret the data:

Make a claim that answers the scientific question.

What evidence was used to write your claim? Reference specific parts of the table or graph.

Explain your reasoning and why the evidence supports your claim. Connect the data back to what you learned about how a bluefin killfish's genes and environment may affect their color.

Name____________________

Did the data support one, both, or either of Becky's two alternative hypotheses? Use evidence to explain why or why not. If you feel the data was inconclusive, explain why.

Your next steps as a scientist: Science is an ongoing process. What new question do you think should be investigated? What future data should be collected to answer this question?