## THE VIRTUAL EVOLUTION STICKLEBACK LAB

As you complete each part of the virtual lab, answer the questions below in the space provided.

## QUESTIONS

INTRODUCTION

1. Define "model organism."
2. How do spines protect ocean stickleback fish?
3. How did ancestral populations of ocean-dwelling fish come to live in freshwater lakes?
4. Watch the video about pelvic reduction in freshwater stickleback. The loss of stickleback pelvic spines is similar to the loss of which body parts in some other four-legged vertebrates?
5. From a researcher's perspective, what is the benefit of having access to hundreds of postglacial lakes?
6. Watch the video with evolutionary biologist Dr. Michael Bell.

Why is the threespine stickleback a model organism for studies in evolution? (List at least two reasons.)
7. Watch the video with Dr. David Kingsley explaining hind-limb reduction. Name two other vertebrate animals whose evolutionary histories included the loss of hind limbs.

## OVERVIEW

1. Click on the interactive stickleback fish. Describe where its spines are located.
2. What is the difference between marine, sea-run, and freshwater stickleback fish populations? Be specific.
3. Watch the video about the stickleback fish armor.
a. In addition to the spines, what is another component of the "armor" of a stickleback fish?
b. Explain how the stickleback armor protects the fish from some predators.

## TUTORIAL 1

1. Describe the following structures:
a. Complete pelvis:
b. Reduced pelvis:
c. Absent pelvis:
2. Start Tutorial 1. Continue to practice scoring fish until you have mastered the technique; then proceed to Experiment 1.

## EXPERIMENT 1

1. Explain in your own words the overall objective of Experiment 1.
2. Click on the link to the map of Alaska, then click on the blue pin " $A$ " on the larger map. What lake is located between Bear Paw Lake and Frog Lake just to the north?
3. In a population, what happens to organisms that are better adapted to the environment in which they live?
4. In the virtual lab window, complete Part 1: Staining the Fish.
a. Why do you think it is important to empty the used stain, destaining solution, and water under a fume hood?
b. How were the fish you will be using in this virtual lab caught?
5. In the virtual lab window, proceed to Part 2: Scoring the Fish.
6. Before scoring the fish, watch the short video on Bear Paw and Frog Lakes. According to Dr. Bell, what is an important difference between Bear Paw Lake and Frog Lake?
7. In the window on the right, click on the link to read more about random sampling. Why are random samples, rather than entire populations, used in most research studies?
8. What is one advantage of studying larger-sized samples?
9. Give an example of sampling bias.
10. Complete Part 2 of the lab in the window on the left.
11. Why is it important that the labels included in specimen jars be made of special paper that does not disintegrate in alcohol over time?
12. Examine the pelvic score data you just collected. Does the pelvic phenotype differ between Bear Paw Lake and Frog Lake fish? Explain.
13. Complete the graphing exercise as instructed by your teacher. After graphing, verify your data. How do your data compare to those obtained by Dr. Bell and colleagues?
14. Complete the Experiment 1 Quiz. Take time to read the explanation for each correct choice provided after each question.
15. Explain why the stickleback fish in Frog Lake are more similar to ocean and sea-run stickleback than they are to the stickleback fish in Bear Paw Lake.
16. In addition to predators, what other environmental factors might be responsible for the differences between Bear Paw Lake and Frog Lake stickleback populations? How would you test your prediction?
17. After completing the quiz, click on Experiment 1 Analysis.
18. For this analysis, what is your null hypothesis?
19. Complete the following tables as you perform the chi-square calculations in the lab.

| Phenotype | Observed (o) | Expected (e) | (0-e) | $(0-e)^{2}$ | $(0-e)^{2} / \mathrm{e}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | Total $=$ | Total $=$ |  |  | $\mathrm{X}^{2}=$ |

Frog Lake

| Phenotype | Observed (o) | Expected (e) | (o-e) | (o-e) ${ }^{\mathbf{2}}$ | $\left(\mathbf{o}^{(0-e)^{2} / \mathbf{e}}\right.$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |  |
|  |  |  |  |  | $x^{2}=$ |

Morvoro Lake

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(\mathbf{o - e})^{\mathbf{2}}$ | $(\mathbf{0 - e})^{2} / \mathbf{e}$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | Total $=$ | Total $=$ |  |  | $x^{2}=$ |

20. For each chi-square calculation, how many degrees of freedom are there? $\qquad$
How did you arrive at this number?
21. What are the $p$ values for the data from each lake?
a. Bear Paw Lake $\qquad$
b. Frog Lake
c. Morvoro Lake
22. Do you "reject" or "fail to reject" the null hypothesis for the data from each lake?
a. Bear Paw Lake $\qquad$
b. Frog Lake
c. Morvoro Lake $\qquad$
23. Complete the Chi-Square Analysis Quiz. Take time to read the explanation for each correct choice provided after each question.
24. Explain what it means to reject the null hypothesis.
25. Explain what it means if the null hypothesis cannot be rejected.
26. Explain the difference between the results of the chi-square calculations for Bear Paw Lake and Morvoro Lake.
27. What do you think might be an explanation for why Morvoro Lake contains fish with and without pelvic spines?

## TUTORIAL 2

1. What score would you assign to a fossil specimen that has only one pelvic spine visible?
2. A stickleback fossil may show no signs of pelvic structures. What are possible sources of error associated with scoring the pelvis of such a fossil as "absent"?
3. Start Tutorial 2. Continue scoring the fossil specimens until you are confident in your scoring abilities; then proceed to Experiment 2. (Most people find that it is more difficult to accurately score fossils than the preserved fish specimens. You may have to practice on 20 specimens or more before you feel you have mastered the technique.)

## EXPERIMENT 2

1. In your own words describe the overall objective of Experiment 2and explain what the data you collect will allow you to estimate.
2. Watch the short video on the Truckee Formation in Nevada. What does each sedimentary rock layer of the Truckee Formation represent?
3. What is one type of information that researchers can gain from studying fossils that they cannot obtain from living populations?
4. Begin the experiment in the window on the left. Complete Part 1: Preparing Fossils. (You may skip this step by clicking on "Skip Part1: Fossil Prep.")
5. Proceed to Part 2: Scoring Fossils. In this virtual lab, you will graph fossil data from six rock layers. Approximately how many years apart are any two adjacent samples?
6. You will collect data on pelvic structures using fossils from rock layers 2 and 5 . Approximately how many years of deposition separate these two layers?
7. Which layer is older, 2 or 5? Explain your answer.
8. Complete Part 2 of the lab in the window on the left. Based on the pelvic phenotypes you measured, do the fossils in layer 2 differ from those in layer 5? Explain how.
9. Complete the graphing exercise as instructed by your teacher. After graphing, verify your data. How do your data compare to those collected by Dr. Bell and colleagues?
10. Complete the Experiment 2 Quiz. Take time to read the explanations for each correct choice provided after each question.
11. What can be inferred about the presence or absence of predatory fish when the Truckee Formation was a lake? Describe the evidence.
12. After completing the quiz, click on Experiment 2 Analysis.
13. Complete the tables below as you perform the rate calculations. (The link to the instructions is very helpful.)

| Sample Layer | Number of Fish with a <br> Complete Pelvis | Total Number of Fish <br> Sampled | Relative Frequency of <br> Complete Pelvis Trait in <br> Population Sampled |
| :---: | :---: | :---: | :---: |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |
| 5 |  |  |  |
| 6 |  |  |  |


| Time | Decrease in Percentage of <br> Complete Pelvis Trait per <br> Thousand Years <br> (Rate of Change) |
| :---: | :---: |
| First 3,000 years <br> (Layer 1 to Layer 2) |  |
| Next 3,000 years <br> (Layer 2 to Layer 3) |  |
| Next 3,000 years |  |
| (Layer 3 to Layer 4) |  |
| Next 3,000 years |  |
| (Layer 4 to Layer 5) |  |
| Next 3,000 years |  |
| (Layer 5 to Layer 6) |  |
| Total 15,000 years |  |
| (Layer 1 to Layer 6) |  |

14. What does it mean when the rate of change is a negative number?
15. Complete the Analysis Quiz. Take time to read the explanation for each correct choice provided after each question.
16. Describe the trend in the data over time.
17. Why is it important to calculate the rate of change over time?
18. In what way is the change in the complete pelvis phenotype in the fossils from the Nevada lakebed similar to what might have occurred in Bear Paw Lake from Experiment 1?

## EXPERIMENT 3

1. In your own words describe the overall objective of Experiment 3.
2. What is one function of the Pitx1 gene?
3. Watch the video segment from the HHMI short film, "Evolving Switches, Evolving Bodies."
a. What is the relationship between changes in body form, the process of development, and genes?
b. Why did Dr. Kingsley do genetic crosses with stickleback fish?
4. In the virtual lab window, click on the blue gloves and perform Part 1: Staining the Fish. You can skip the staining procedure if you already completed it in Experiment 1.
5. Proceed to Part 2: Scoring Pelvic Asymmetry. Which one of the three pelvic girdle phenotypes is analyzed in more detail in this experiment?
6. What is the difference between left-biased and right-biased pelvic asymmetry?
7. In the virtual lab window, complete Part 2: Scoring Pelvic Asymmetry.
8. Complete the graphing exercise as instructed by your teacher. After graphing, verify your data. How do your data compare to those collected by Dr. Bell and colleagues?
9. Complete the Experiment 3 Quiz. Take time to read the explanation for each correct choice provided after each question.
10. The pelvic asymmetry observed in stickleback fish from Bear Paw Lake and Coyote Lake is biased toward which side?
11. Based on previous research conducted in mice, what does this observed bias among Bear Paw Lake and Coyote Lake stickleback suggest about the genetic mechanisms of pelvic reduction in these two populations?
12. After completing the quiz, click on Experiment 3 Analysis.
13. For this analysis, what is the null hypothesis?
14. Complete the following tables as you perform the chi-square calculations in the lab.
Bear Paw Lake

| Phenotype | Observed (o) | Expected (e) | (o-e) | $(\mathbf{0}-\mathbf{e})^{2}$ | $(\mathbf{0}-\mathrm{e})^{2} / \mathbf{e}$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  | Total $=$ | Total $=$ |  |  | $x^{2}=$ |

Coyote Lake

| Phenotype | Observed (o) | Expected (e) | (o-e) | (o-e) ${ }^{\mathbf{2}}$ | $\left(\mathbf{o}^{(0-e)^{2} / \mathbf{e}}\right.$ |
| :--- | :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |  |
|  |  |  |  |  | $x^{2}=$ |

15. For each chi-square calculation, how many degrees of freedom are there? $\qquad$
16. What are the $p$ values for the data from each lake?
a. Bear Paw Lake $\qquad$
b. Coyote Lake $\qquad$
17. Do you "reject" or "fail to reject" your null hypothesis for the data from each lake?
a. Bear Paw Lake $\qquad$
b. Coyote Lake $\qquad$
18. Complete the Chi-Square Analysis Quiz. Take time to read the explanation for each correct choice after each question.
19. What does it mean to reject the null hypothesis for Bear Paw and Coyote Lakes?
20. Complete the Final Quiz. You may print and/or email the Progress Report page to your teacher, be sure to enter your name in the box in the upper right corner.

## AUTHOR

Ann Brokaw
Rocky River High School
Rocky River, Ohio

