Goals

By the conclusion of this lab, you should understand some very basic statistics and be able to use them in the course of a scientific investigation. You should also be able to collect data on organisms and use a suitable program to make graphs and tables.

Background

In this lab you will be examining specimens of threespine stickleback (*Gasterosteus aculeatus*) from different lakes in Alaska. Freshwater populations of threespine stickleback provide an excellent opportunity to study rapid and dramatic evolutionary responses to natural selection. Ancestrally, all lake populations have evolved from a large marine anadromous (meaning, swims to freshwater to breed) population. All of the lake populations you will examine today must have evolved from this marine population within the past 22,000 years, as glaciers glaciers covered the area before then. A drawing of a marine stickleback is below in Figure 1.

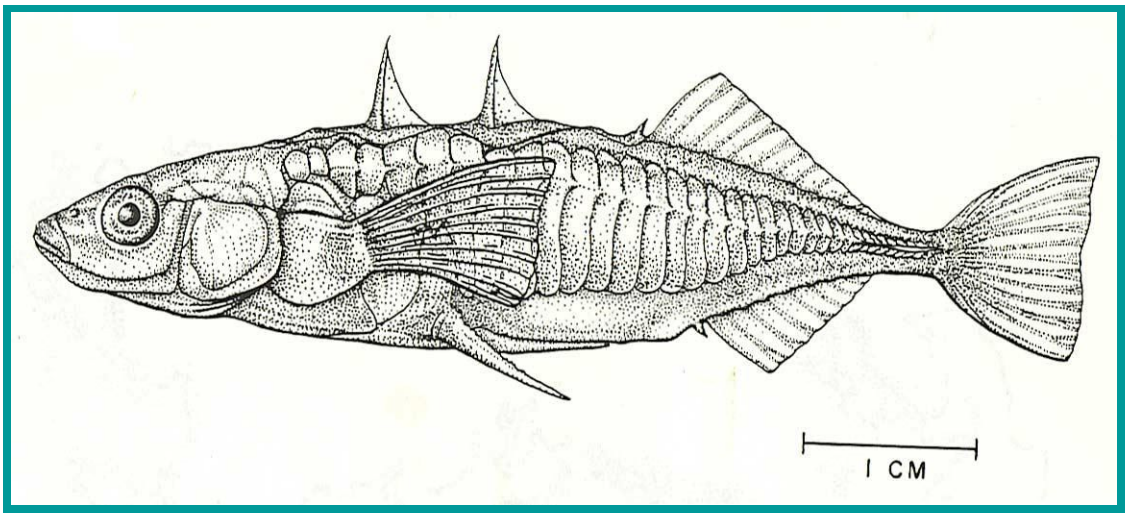

Figure 1: Example of a marine stickleback (courtesy of Dr. Matthew Travis, Rowan University)

Freshwater populations of stickleback look quite different from this marine individual, and many freshwater populations also differ from each other. For example, Figure 2 shows some of the differences that exist between freshwater populations, as well as between freshwater and marine populations. The marine population is in the center, and several different freshwater morphologies are arrayed around the outside. In this lab, you will examine fish from different populations, learn some ecological differences between the lakes, generate hypotheses as to how the ecology might affect the fish, measure several traits

on the fish, and summarize these measurements using a combination of visualization and summary statistics.

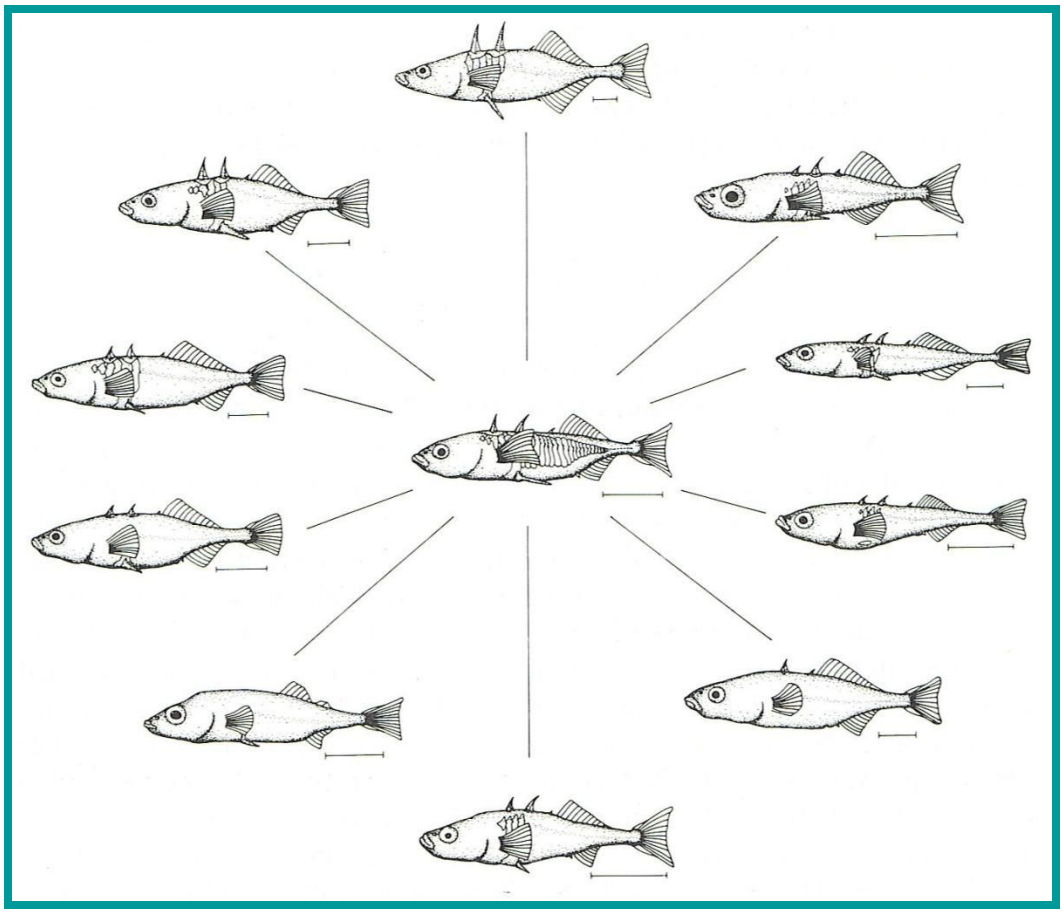

Figure 2: Adaptive radiation of freshwater threespine sticklebacks from a marine ancestor (Bell and Foster, 1994)

Familiarizing yourself with the threespine stickleback

1. Collect and examine a sample of marine threespine sticklebacks. These fish have been stained with a chemical dye called Alizarin red, which binds to bone. Therefore, any red structure you see on the fish that is stained red is made of bone.

   Think: What are your initial observations about these fish?

2. Consider the three morphological traits shown in Figure 3. The first of these is the armor plates that run down the entire side of the body, from just behind the skull to the caudal fin (tail).

   Think: What do you think the function of these plates might be and why?

3. Next examine the dorsal spines on the back of the fish. There are 3 spines (hence the species' name), each of which can be raised or lowered by the fish. Gently raise and lower the spines (be careful not to stick your fingers! The spines are **SHARP**).

   Think: What do you think the function of the dorsal spine might be and why?

4. Now explore the pelvic girdle and spines. This complex of bones is homologous with the limb girdles in other vertebrates (including humans). The pelvic girdle consists of three main parts, as shown in Figure 3.Make sure you can identify all three parts of the pelvic girdle on the fish. Like the dorsal spines, the pelvic spines can be raised and lowered by the fish. Gently raise the pelvic spines. Notice that they lock in place. To lower them again, press down on them NEAR THE BASE OF THE SPINE. If you push down near the tip of the spine you could break the spine (and also stick yourself).

   Think: What do you think the function of the pelvic girdle might be and why?

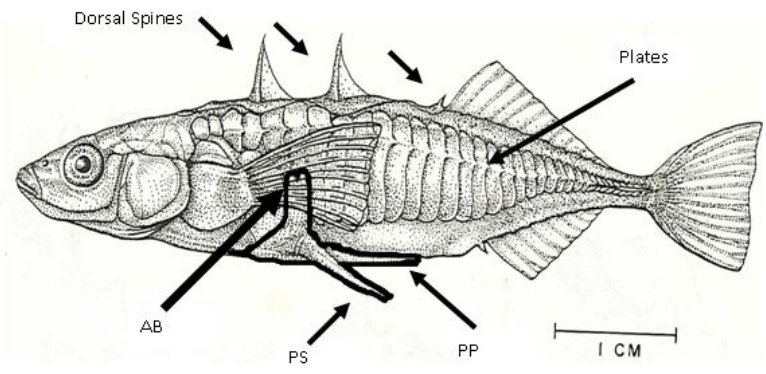

Figure 3: Marine stickleback with key morphological features. Labeled elements of the pelvic girdle include AB: ascending branch; PP: posterior process; and PS: pelvic spine. (courtesy of Dr. Matthew Travis, Rowan University)

5. Obtain specimens from two of the populations (each jar comes from a different lake or from the ocean). ***IT IS EXTREMELY IMPORTANT TO KEEP FISH FROM DIFFERENT POPULATIONS SEPARATE*** so that you don't mix them up!

   Think: What differences do you notice between the lake populations and the marine population? Why do you think that these differences are present?

Methods: Making Measurements

Three traits in which stickleback populations often differ include the following:

- The number of lateral plates
- Body depth
- Pelvic girdle expression

In this lab, you will collect data on these three measurements and compare the populations in terms of these traits.

Measuring the number of lateral plates

Lateral plates are located on the side of the body beginning just behind the head of the fish. Simply count the number of plates you see. The first few may be very small, and a dissecting microscope may help you see them. The number of plates can differ on the left and right side of the body, so in order to make sure every group is measuring the same thing, count only the number of plates on the left side of the fish. Record your data in the appropriate table below.

Measuring body depth

Body depth can be measured with calipers. Measure body depth just in front of the first dorsal spine (the one closest to the head of the fish). Take your measurement when the calipers just touch both sides of the body. Do not squeeze the calipers - this will yield an inaccurate measurement! Record the body depth of the fish in the appropriate table.

The calipers we have make measurements in millimeters. Note the scale along the ruler part of the calipers (see above, to the right of the dial). If you're using a set of calipers with a dial, they generally tell you what the smallest division is on the dial. For example, if it says "0.1 mm" on the dial, that's the distance between the smallest tick marks on the dial. Most of the dial calipers we have show 10 mm with one full rotation of the "hand" around the dial. Make sure this

is the case by watching how far the dial moves along the ruler as the "hand" makes one full rotation.

Measuring pelvic girdle expression

To measure pelvic girdle expression we will use the so-called Pelvic Score. This is a categorical measurement of the size and complexity of the pelvic girdle. The scoring system is diagrammed below in Figure 4. Ventral means the fish is upside-down and you are looking at the belly. Lateral means you are looking at the side of the fish.

Table 1: Pelvic Score categories

Pelvic score	Description of pelvic girdle
0	Completely absent
1	Small oval of bone
2	The oval plus the ascending branch (AB)
3	All the elements of a Pelvic Score of 2, plus the posterior process (PP) is present
4	All the elements of a Pelvic Score of 3, plus the pelvic spine is present

Give each fish a score of 0 - 4 *for each side of the body* and then sum the score to find the total Pelvic Score. If all three parts of the pelvic girdle are present on the left, it is scored a 4, and if all are present on the right, it is scored a 4. The total score for that fish would be 8. Record your data in the appropriate table below.

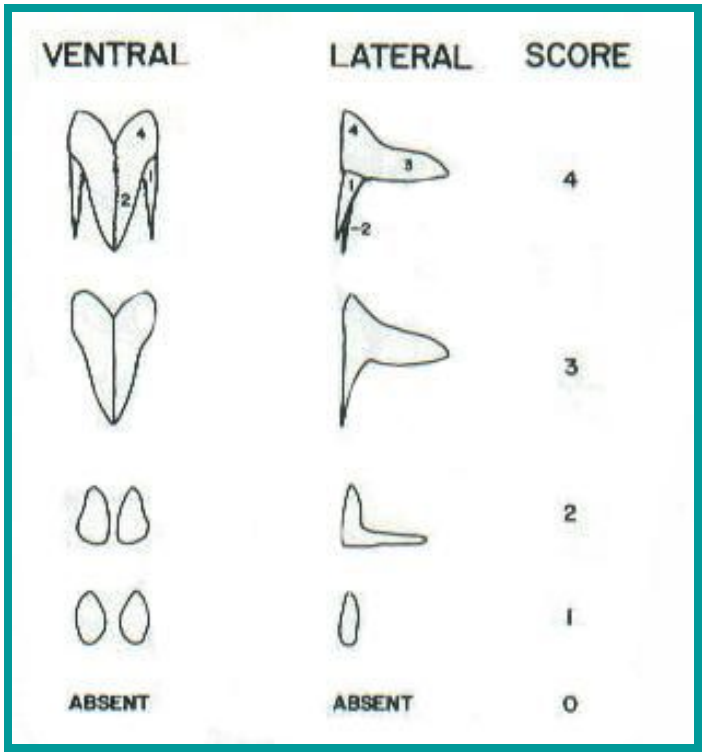

Figure 4: diagrams of the pelvic girdle for each Pelvic Score level. Both ventral and lateral views are shown (from: Bell et al., 1993).

Analyzing your data

1. Using either Excel or the Shiny Plot application, make a figure to represent each of the following measurements:
    - The number of plates on the *left* side
    - The body depth
    - The body length
    - The pelvic score

    You should visualize each of these measurements *across stickleback populations*. You may choose the type of figure to make as long as it is appropriate for the type of data you are visualizing. (Hint: You are measuring quantitative data and will show these distributions across populations, which is a categorical variable).

2. Using either Excel or the Shiny Plot application, make a figure to show the *relationship* between each of the two pairs of measurements. Note there is no specific dependent and independent variable here, so you are free to choose which variable should go on which axis:
    - Body length vs body depth
    - Number of plates vs pelvic score

    For each figure you make (a total of six), provide a 2-3 sentence interpretation of the figure. The interpretation should simply explain what you see - you should not speculate about fish evolution of function; just discuss what the plot shows.

Analyzing your data

Using Excel, determine the *mean*, *standard deviation*, and *standard error* for each variable you collected.

What should I submit?

On Blackboard, you will submit two items: your Excel spreadsheet (this will be the same for a given lab group) and a typed document (written in your own words). Please see the template for each of these documents in Blackboard. *To receive full credit you must use the templates provided and follow all instructions carefully!*

Acknowledgements

This lab was modified from one designed and written by Dr. Matthew Travis, of Rowan University's Department of Biological Sciences in 2008, and further revised by Dr. Courtney Richmond of Rowan University's Department of Biological Sciences. Dr. Travis collected the threespine sticklebacks from these Alaskan Lakes in the summer of 2008.

References

Bell, M.A. and Foster, S.A. 1994. The evolutionary biology of the threespine stickleback. Oxford University Press, Oxford, UK.

Bell, M. 1993. Stickleback Lake Information. unpublished data, compliments of Dr. Matt Travis, personal communication. October 2009. Rowan University.