ANOVA: Evolution in Fossil Stickleback

***Complete the exercise, type your answers to the questions at the end, and submit your report via moodle within the week of this lab**

For this lab you will be measuring two traits on fossil threespine stickleback and determine whether the fish evolve over the time span of this sequence using ANOVA. The fossils come from a 10 million year old lake deposit in Nevada. The conditions of this lake were such that every year, there was a spring and fall diatom bloom. As these diatoms died, they sank to the bottom of the lake, creating a sediment layer that eventually turned into a rock called diatomite. This continuous process of deposition resulted in a year by year record of deposition for over 120,000 years that can be documented by counting the layers in the rock. When the stickleback that lived in this lake died, they also sank to the bottom, and were preserved mostly intact. When fossils were collected from this deposit, we intensely sampled a small temporal window and pooled the fossils from that time frame to create a sample. Samples were made approximately 1000 years apart for 21,000 years. You will be measuring two traits on these fish from three of these samples (0, 3500, and 7500).

Access to the fossil images

Go to Dr. Takahashi's public space (mt027). There you will find folders "Stickleback_Images" with digital images of fossil stickleback from 3 different times during the fossil sequence. The times begin at an arbitrary 0 and go forward from there. In each folder, you will find 10 images on which you will measure phenotypic traits.

Traits

You will be measuring 2 traits on the fish: the length of the second and third dorsal spines (the two closest to the tail of the fish). The program we will use to measure the fish is called ImageJ, and is freely available on the web from NIH. You can install this to your own computer or use the University computers.

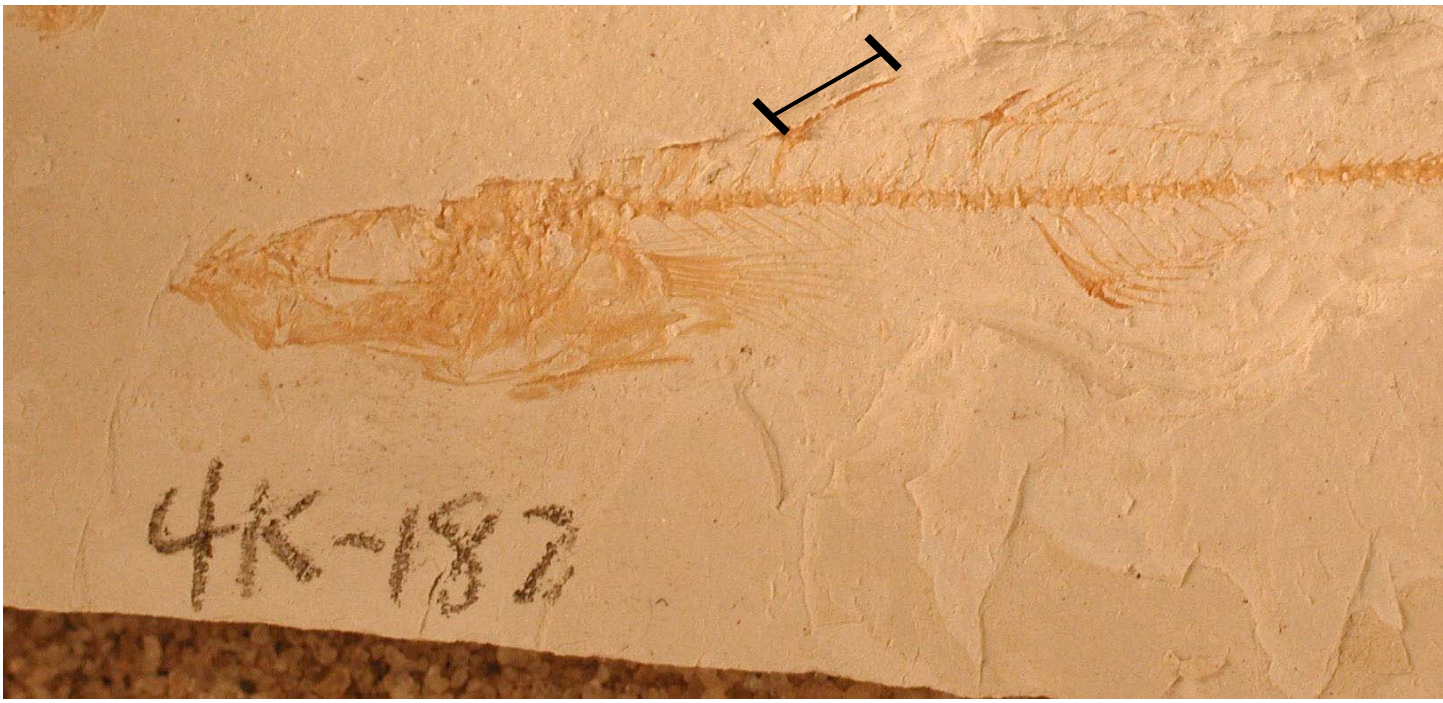
Directions for using ImageJ

1. Open the program. To open your first image, go to File → Open. A pop up window will open and select the first folder of stickleback images. Open the first one.
2. Once the image opens in a new window, you will need to have a reference length. You will notice in the image that there is a ruler alongside the fossil. Click on the "straight line selection" box at the top of the ImageJ window. Go to the image and click the mouse button on a line on the ruler. This will anchor a point on the image and draw a line from there to wherever you move the cursor. Click again on a line on the ruler 10mm away from your first point.

Now go to the Analyze menu at the top of the ImageJ window. Go to "Set Scale". Another window will open. Enter "10" in the "Known Distance" box. This will convert the distance you measure in pixels to millimeters. Do NOT check the Global box, as each image was taken at a

slightly different distance from the camera lens. Click "OK". Make sure to do this every time you measure a new image

3. Now you are ready to measure the length of the spines. Using the straight line tool again, measure the distance from the base of the spine to the tip of the spine (see below for sample, this is a second spine) by clicking at the base of the spine and then clicking again at the tip. Go to the Analyze menu again and click "Measure". A new window will open with the measurement of that length in mm. Enter this number in an Excel spreadsheet (see the example table below). Do the same for the third spine. Read the next step before you go to the next image!



4. We want to do one more thing before we are done with that image. Measure the length of the fish from the tip of the head to the last vertebrae. This measurement is called standard length and will be used to adjust your spine lengths by the size of the fish. (Think about why we want to do this). You should now have four columns in your spreadsheet, one for the number of the fish, one for the length of the second spine, one for the length of the third spine, and one for standard length.

5. It is now time to measure the spines on the next fish. Go back to the ImageJ menu and click→ File Open Next. The next image should open. Set the scale again, and repeat the previous measurements on this fish. Continue until all fish have been measured.

Data Analysis

1. In your Excel Spreadsheet, divide the length of each spine by the standard length of the fish. In a new workbook, set up the spine length data to run your two analyses of variance (one for each spine)

2. Set up your Excel sheet to run the ANOVA. You should have 3 samples for each of the two spine lengths. To set up the test, you will put 3 columns side by side in the spreadsheet as shown below (this is the final set up. You need to create a few leading tables for data recording).

2nd spine	0	3500	7500
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

3rd spine	0	3500	7500

3. Microsoft Excel 2010 comes with statistical package that has ANOVA. Go to "Data" tab, click on "Data Analysis", and choose "Anova: Single Factor". Follow the guidance to test whether there is an overall difference in spine length among the three groups. If your excel does not have "Data Analysis" tab, talk to your instructor or TA.

4. Use the same procedures you used in Part I of the lab to conduct post-hoc pair-wise tests.

Questions

1. Why do you think we divided the spine length by body length?

2. What are your alternative and null hypotheses about differences in spine lengths among the three groups? Explain why you think this.

3. Do spine lengths differ among samples in this fossil deposit? Report your ANOVA results including post-hoc Tukey tests and interpret them.

4. Which means differ from the others? Report your post-hoc test results and interpret them.

5. What might explain the pattern exhibited in these data? (i.e. natural selection, drift, etc). Explain your answer and see if you can find evidence for this in the scientific literature. Make sure you cite the paper you found by using the format of journal *Evolution* (http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1558-5646).