

ECOLOGY and EVOLUTION

Week 1: August 29 – September 2, 2011

Stickleback fish and *Bombina* tadpoles:

Basic computational skills for future work with variation

Welcome to your first introductory biology laboratory. From lab lecture you learned that today is about getting comfortable and beginning to tease apart the skills required to see you through the year as a working scientist. It will also allow you to shake out the cobwebs of summer and gauge your personal velocity relative to the amount of required work. With a lot of extra time today, many of you will finish early. Lab work should never be a race, but you will often be spending 4 hours in each lab.

PLEASE READ the entire lab manual before coming to lab and ask us as many questions as you like.

Be sure to have your Bio Binder and Lab notebook with you.

Today, you will be introduced to two vertebrate model organisms that you will be discussing and working with repeatedly through the first 1/3 of the semester. You will also become familiarized with laboratory computers and analysis via three documents in the Bio Binder: Computer Basics D, Measuring with **ImageJ** E, and **JMP** Instructions G.

Specifically, you will become familiar with:

- 1) A fish that lives in Reed Canyon, the threespine stickleback, *Gasterosteus aculeatus*, and the amphibian larvae, *Bombina orientalis*, that we have worked with for years in the field in the Republic of Korea.
- 2) Our new Intel Mac's and their connections to servers, printers, and some of their specialized biology software.
- 3) **JMP** statistical analytical software by collecting your own data from these two organisms and creating graphs and testing hypotheses.

1. Introduction to the Animals via the Encyclopedia of Life *(Please complete A and B before coming to lab this week. Your lab notebook is a good place to take notes as you go.)*

This handout is accessible as a document from the **Courses Server** using **AFS** (see Bio Binder page D-2). Lab 1 quant skills F2011.pdf

Clicking on the web links is easier than typing them in.

A. Sticklebacks

The threespine stickleback (*Gasterosteus aculeatus*) is fast becoming an important model organism for evolutionary, developmental, and genetic studies. This is primarily due to the fact that the fish has long been a well-known study organism from a behavioral and ecological perspective and its genome is nearly complete in DNA sequence.

We started working with stickleback in our Intro. Bio. classes here in the Reed Canyon (<http://web.reed.edu/canyon/>) in November 2005. There are also two independent student projects (Nick Manoukis '95, Ezra Lencer '05) and four senior theses (Meng Qi '05, Max Press '08, Annika Saltman '08, and Laila Bryant '09). This year in lab, we are going to gather and analyze data, test hypotheses, and add to our long-term data base as we track our populations in the canyon.

For now, go to the Encyclopedia of Life website (<http://www.eol.org/pages/1>). This is a new and valuable site, conceived of by the great Dr. E. O Wilson of Harvard and developing through collaborations of the major biodiversity institutions and museums of the world. Navigate in as follows:

Kingdom Animalia -> Phylum Chordata (chordates) -> Class Actinopterygii (the ray-finned fish group) -> Order Gasterosteiformes (dragonfishes)-> Family Gasterosteidae (sticklebacks and tubesnouts) -> Genus *Gasterosteus* -> Species *Gasterosteus aculeatus* (three spine stickleback). **You do not have to memorize these.** But, play with the pictures. In the process, you will see how diverse the fishes are.

For more detailed information on sticklebacks, check out Susan Foster's link at <http://www.clarku.edu/activelearning/departments/biology/foster/fosterD.cfm.html>. We will be referring to this link again later in the semester.

Questions you must know how to answer using the Encyclopedia of Life:

1. What are the three most distinctive morphological features of chordates?
(Find the answer in the Encyclopedia of Life (under chordates)).
2. What is a current estimate of numbers of known and living species of ray-finned fish?

B. The fire-bellied toad - *Bombina orientalis*–

Like stickleback fish, amphibians are vertebrates, and we have the expertise to make them easy to handle. For an example of the latest research published by the Kaplan Lab, check out the pdf on the **Courses Server** or go to the following link

<http://academic.reed.edu/biology/professors/rkaplan/docs/Kaplan&Phillips, 2006.pdf>.

In addition, go to the Reed Library Home Page (<http://library.reed.edu/>). Search for *Bombina* in the Reed College Library. On the left, under **Content**, select “**Thesis/dissertation (32)**”. Note approximately 32 Senior theses by Reed College students are the first items to appear.

Now go back to the Encyclopedia of Life at <http://www.eol.org/pages/1>.

Navigate in as follows:

Kingdom Animalia (animals) -> Phylum Chordata (chordates) -> Class Sarcopterygia (lobe-finned fish and terrestrial vertebrates) -> Class Amphibia (amphibians) -> Order Anura (frogs and toads) (Amphibia with -> Family Bombinatoridae -> Genus *Bombina* -> Species *Bombina orientalis* **You do not have to memorize these.**

Using the AmphibiaWeb website (<http://amphibiaweb.org/>) browse by family.

As with stickleback, we will not have lab time to spend appreciating the details of tadpole morphology, physiology, and behavior, nor the theory and science behind the motivation for the experiment that we will set up next week. We will develop much of this in lecture.

To learn about anuran larvae, link to [Tadpoles of the United States and Canada: A Tutorial and Key](#) by Ronald Altig, Roy W. McDiarmid, Kimberly A. Nichols and Paul C. Ustach. <http://www.pwrc.usgs.gov/tadpole/tutorial.htm>.

Questions you must know how to answer:

1. How many major groups of living amphibians are there worldwide? (from Encyclopedia of Life).
2. How many species of living frogs, salamanders and caecilians are there? (from Amphibia Web; search the database, then about amphibians, then species numbers)

3. 2. A comparative study of two populations of stickleback in the Reed Canyon - testing univariate differences with Analysis of Variance (ANOVA)

Find the two sample sites (Reed Lake and Ritmanis Pond) on the map below (Fig.1). We will be sampling these sites during weeks 3 and 4. You can see live fish in two tanks at the back of the lab today.

Hypothesis that we will test today from digitized photos (in question form as opposed to null hypothesis form):

Is there a difference in the size of stickleback in the Reed Lake versus the Ritmanis Pond, as determined by a random sample of 5 fish from each location in February of 2005?

(See Bio Binder Quan. Exp. Design F-2 – What is a Hypothesis?)

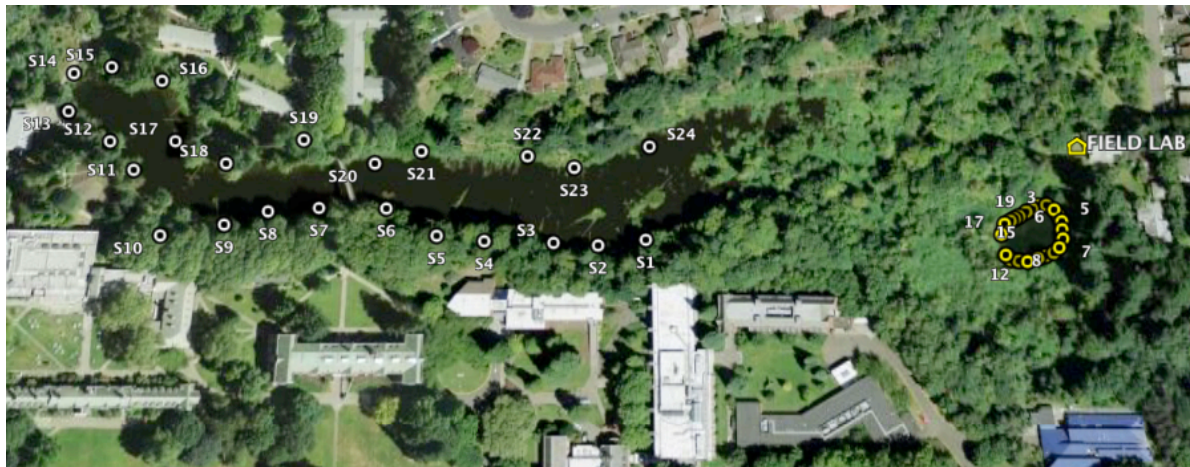


Figure 1. Reed Lake (Left) – Stickleback survey sites – Ritmanis Pond (Right)

1. Work in **PAIRS** on the computer. **Log in** and go to the **Courses Server** (see Bio Binder Computer Basics D-1+2).

Courses Server/Biology/Bio101 102 Intro Biology/1st Ecology&Evolution-Kaplan F11/ Laboratory Materials/Lab 1 Quantitative Skills

2. Drag to your **Desktop** two folders called **Reed Lake Stickleback** and **Ritmanis Pond Stickleback**.

These folders contain images of cleared and stained stickleback studied by Meng Qi '05 during the course of her Senior thesis (Qi, 2005). (She cleared away muscle tissue via trypsin digestion and stained the remaining animal with two different stains, one for bone and one for cartilage.)

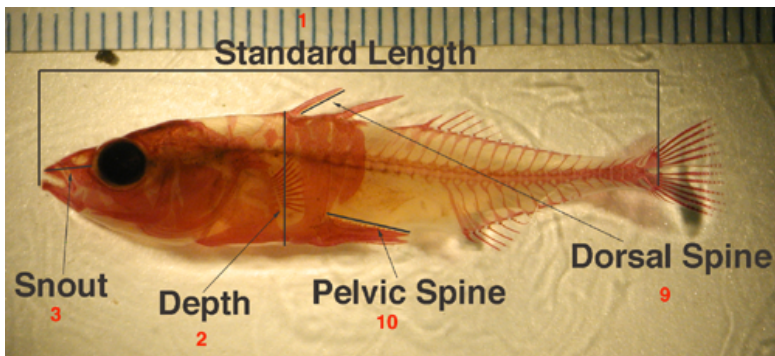


Figure 2. Stickleback fish stained for bone and cartilage. Photo by Lencer '06, 2005. Clearing and Staining by Qi '05, 2005.

3. Drag a file from inside one of the fish folders onto the **ImageJ** icon in the dock.

(See Bio Binder Measuring with **ImageJ** E-1+2 and follow directions. (Reminder from Bio Binder: before measuring the fish, be sure to **Set Scale** [use 20.0 mm] for each photo.)

Drag the mouse from the nose or snout to the beginning of the tail or dorsal spine (as shown in Fig. 2), and you'll see the Standard Length. Do the numbers make sense in relation to the ruler? Most fish are 25-45 mm in standard length.

4. **Record in your lab notebook** the **FishID** and **location** (i.e., whether it is from Reed Lake or Ritmanis Pond). **Measure and record Standard Length** to the nearest 0.1 mm for **5 fish** from Reed **Lake** and **5 fish** from Ritmanis **Pond**.

Analysis

1. Open **JMP** and within it, open a **New Data Table**. (See Bio Binder JMP Instructions G-2.)

2. In this **Data Table**, enter the following 3 column headings:

-Fish ID



-Location

-Standard Length (mm)


Save your **JMP** file with an informative name to your **Desktop**.

Record the file name in your lab notebook.

3. In your open **JMP Data Table**, enter:

-Fish ID (As you enter a letter, notice that the column icon changes from a **blue triangle**  to a **red histogram** . This is appropriate for a categorical variable.)

-Location: Lake or Pond (This is case-sensitive to register as only two categories.)

-Lengths: numbers only, mm units go in the column name (Column must be **Numeric** and **Continuous** as represented by the **blue triangle**  column icon.)

Be sure to **Return** after your last data entry before analyzing.

Save your **JMP** file to the **Desktop** regularly.

Drag from the **Desktop** to your **Home Server** at the end of lab. (See Bio Binder D-2.)

4. See Bio Binder JMP Instructions G-6 for **Frequency Distribution**.

Choose **Standard Length** as your **Y (Response) Variable**.

Print two copies of the **Distribution**, 3-hole punch, and put in your Bio Binders.

5. See Bio Binder JMP Instructions G-9 for **Analysis of Variance (ANOVA)**.

Choose **Standard Length** as your **Y (Response) Variable**.

Use **Location** as **Model Effect** for ANOVA.

Follow the instructions on G-9 until you have something like the output example on G-10.

Presentation

1. In **JMP**, **Select All** and **Copy**, and then **Paste** the graphs and tables into a blank **Word** document.

2. From the **Courses Server**, drag a copy of the **ANOVA report template.doc** file to your **Desktop**, **Open** and **Save As ...** your filename.

To help you learn to write figure legends and table captions and to report statistical results properly, this template models them for you. (See Bio Binder Lab Report Instructions R-3.) You can use this file for your report by replacing the existing graphs and tables with your results and modifying them appropriately.

The following two pages show the original JMP output and the modified form for turning in as your report. Page 10 points out important differences between the two.

3. Indicate what you think the results show with one simple sentence that answers the following question.

Are the fish from the two locations of different size?

If the ANOVA P-value is <0.05 , then you can reject the null hypothesis.

If you can reject the null hypothesis, are the fish longer in the lake or the pond?

If the ANOVA P-value is >0.05 , then you fail to reject the null hypothesis and there is no statistically significant difference in length between the lake and pond fish that you sampled.

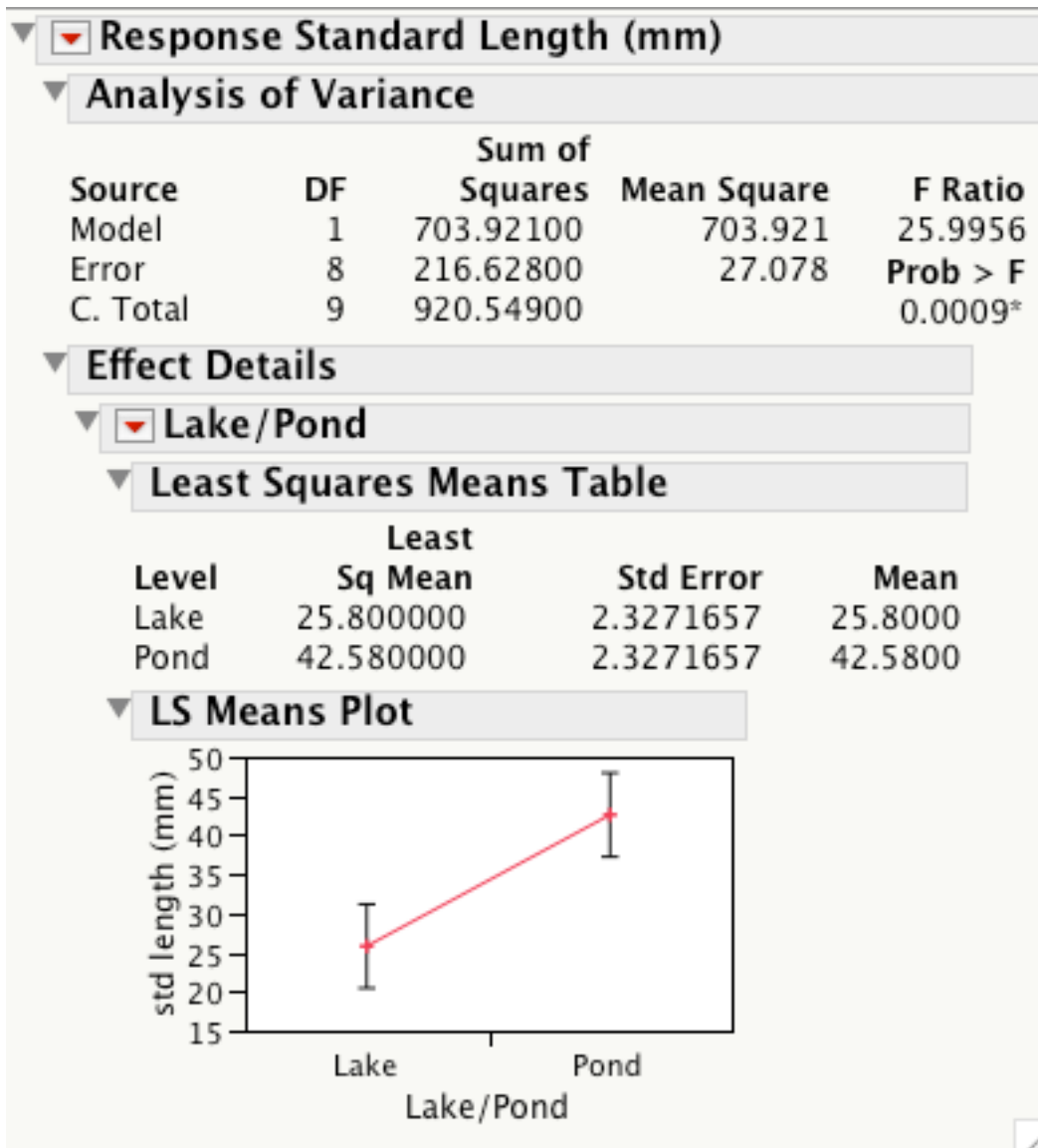
(See Bio Binder Statistics Explained H-6.)

Did other people in the class get the same answer as you?

The null hypothesis you have rejected or not rejected is:

There is no statistically significant difference in standard length between a random sampling of fish in pictures from the lake vs. the pond.

Original JMP ANOVA output looks like this (your numbers will vary).



Please turn in only one copy with both partners' names at the top as in the template.

Extract parts of above JMP output to look like the template on the following page and then turn it in.

Names: Ann Jones, Joe Smith

Mail Stop #1234

Lab day: Monday

Lab instructor: Ned Knight

Date: August 29, 2011

Size comparison of stickleback fish in Reed Lake and Ritmanis Pond

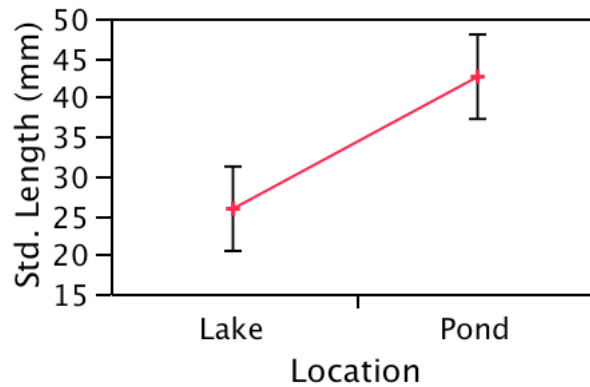


Figure 1. Mean standard length of stickleback fish (*Gasterosteus aculeatus*) in Reed Lake (N=5) vs. Ritmanis Pond (N=5) with 95% confidence intervals

Table 1. Analysis of Variance for standard length of stickleback fish^a in Reed Lake vs. Ritmanis Pond

Source	DF	Sum of Squares	Mean Square	F Ratio	P Value
Location	1	703.9	703.9	26.0	0.0009
Error	8	216.6	27.1		
Total	9	920.6			

^a *Gasterosteus aculeatus*

The mean length for pond stickleback fish (42.6 mm) was statistically significantly greater than the mean length for lake stickleback fish (25.8 mm) (ANOVA, F=26.0, df=1,8, P=0.0009).

Note the differences between the original JMP output and the format to hand in:

- No text goes above the figure.
- Figure legend goes below the figure.
- Table caption goes above the table.
- Numbers in the table have the same number of digits to the right of the decimal place as the original measurements.
- Source of variation has been changed from **Model** to actual source in this case **Location**.
- Lettered footnotes go directly below the table.
- Description of pattern and testing of null hypothesis goes last.
- If your P value was >0.05 , your statement would be more like this:

The mean length for pond stickleback fish (32.2 mm) was not statistically significantly different than the mean length for lake stickleback fish (29.5 mm) (ANOVA, $F=0.2$, $df=1,8$, $P=0.6797$).

- Degrees of freedom (DF) is based on sample size, experimental design, and type of statistical test being done. It is often the sample size -1 or the number of treatments -1.
- For an experiment with this design,
 - Location DF is 2 locations -1 =1
 - Error DF is Total DF - 1 =8
 - Total DF is 10 fish -1 =9
- For ANOVA, it is standard to report the treatment DF (Location in this case) and the Error DF.

Please turn in only one copy with both partners' names at the top as in the template.

3. Analysis of body shape - bivariate analyses with two week old (14 dpf) *Bombina* tadpoles

Below are the two measurements (body length and tail height) that we need to measure using **ImageJ** (Fig. 3).

You can see live tadpoles in tanks at the back of the lab.

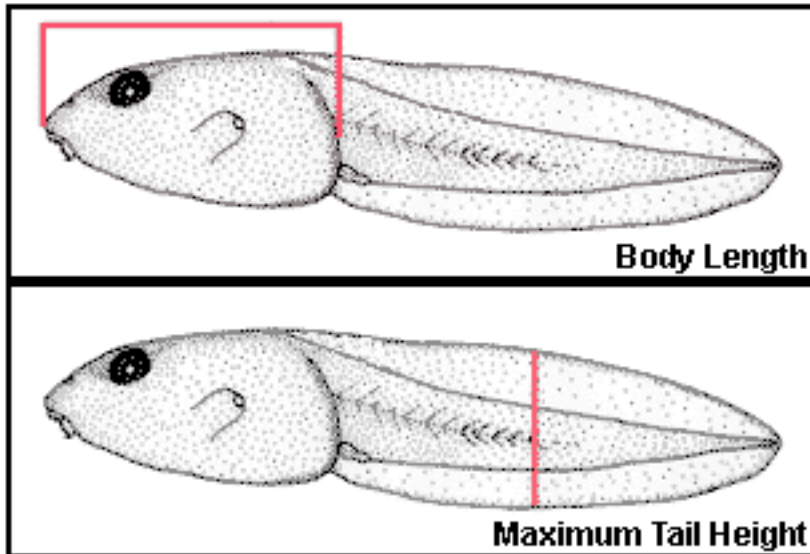


Figure 3. Tadpole morphology. From Altig et al., (1999)

The hypothesis that we will test today from digitized photos (in question form as opposed to null hypothesis form):

Is there a relationship between the maximum height of a tadpole tail and its body length?

1. From the **Courses Server**, drag the folder called ***Bombina* Tadpole Pictures 14dpf** to the **Desktop** (photos by Juliana Arrighi '07). Use **ImageJ** to measure **Body Length** (mm) and **Tail Height** (mm) for each of **10 tadpole** images. Double-check your measurements by looking at the ruler and estimating in mm. Your numbers should be between 2 and 10 mm.
2. Record in your lab notebook, the **Tadpole ID** and measurements of **Body Length** (mm) and **Tail Height** (mm) **paired for each tadpole**. Note in your lab notebook that these tadpoles are 14 days post fertilization (dpf) at 24 °C.

Analysis

1. Open **JMP**, and within it, open a **New Data Table**.
2. In this **Data Table**, enter the following 3 column headings:
 - Tadpole ID
 - Body Length (mm)
 - Tail Height (mm)
 - Give your file an informative name, and **Save** it to your **Desktop**.
 - Record the file name in your lab notebook**.
 - Enter data, and be sure to **Return** after your last data entry before analyzing.
 - Save** your **JMP** file to the **Desktop** regularly.
 - Drag** from the **Desktop** to your **Home Server** at the end of lab.
3. Proceed with **Bivariate Regression Analysis**. (See Bio Binder JMP Instructions G-12.) Put Body Length (mm) on the X- axis and Tail Height (mm) on the Y- Axis.
 - The equation for the straight line that **JMP** drew for you will be under the graph.

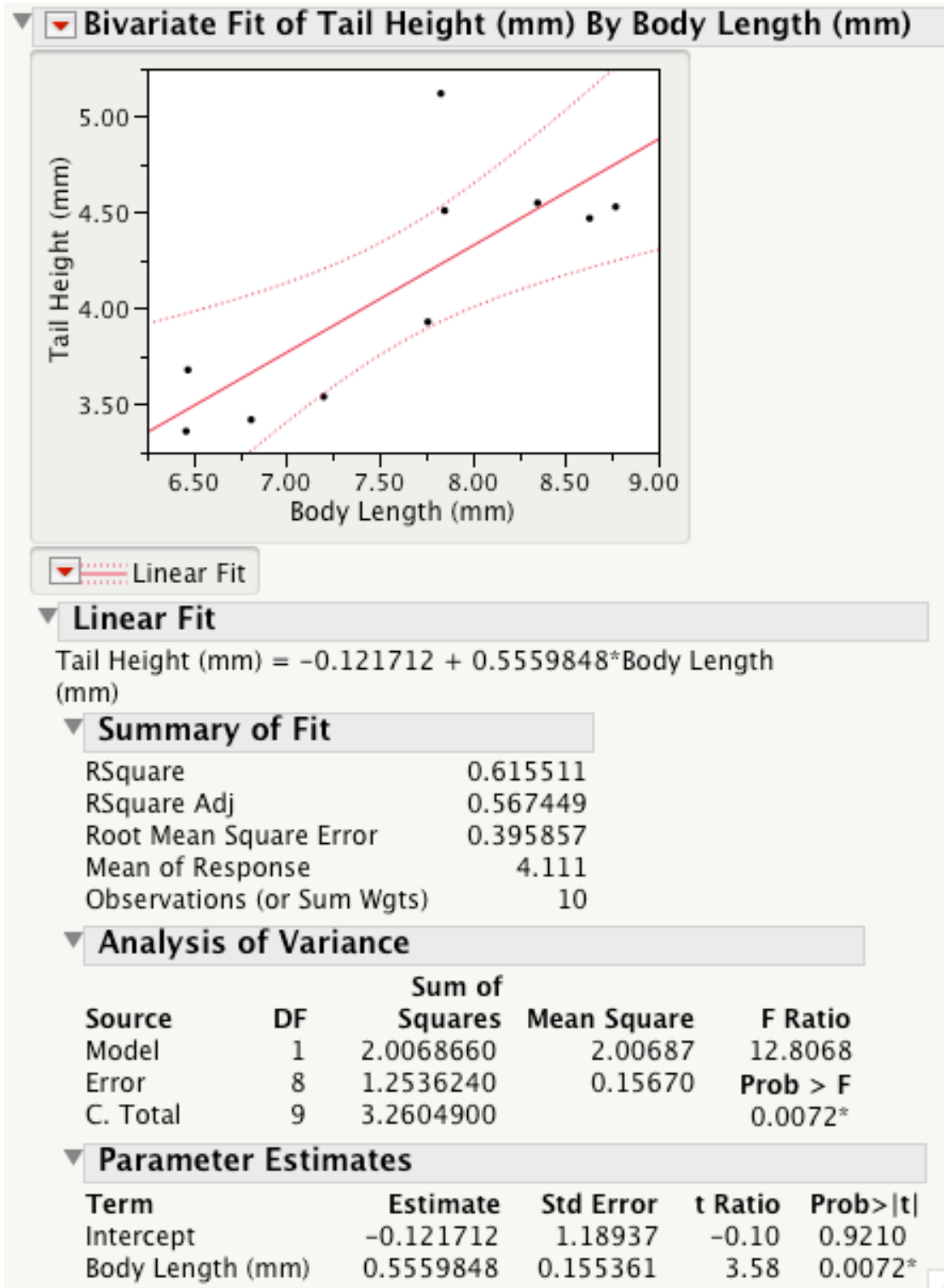
Presentation

1. Once you have your **Bivariate Fit Plot**, double click on the X-axis numbers (not on axis label) and select **Format: Fixed Dec 2**. Do this again to the Y-axis. This will present the axis numbers with the same number of significant figures.
2. **Select All** and **Copy**, and then **Paste** the graph and tables into your **MS-Word** document.
 - From the **Courses Server**, drag a copy of the **Regression report template.doc** file to your **Desktop**, **Open** and **Save As...** your filename.
 - The following two pages show the original JMP output and the modified form for turning in as your report. Page 15 points out important differences between the two.
3. Indicate what you think the graph shows.
 - Does body length help predict tail height in your sample of 10 tadpole pictures?
 - Is the P-value < 0.05 ?
 - Do the 95% Confidence Intervals include a line with a slope of zero?(See Bio Binder Statistics Explained H-9.)

Null hypothesis you have rejected or not rejected is:

There is no statistically significant relationship between body length and tail height among tadpoles in pictures sampled.

Original JMP regression output looks like this (numbers will vary).



Extract parts of the preceding JMP output to look like the template on the following page and then turn it in:

Names: Ann Jones, Joe Smith

Mail Stop #1234

Lab day: Monday

Lab instructor: Ned Knight

Date: August 29, 2011

Bombina orientalis tadpole body shape analysis

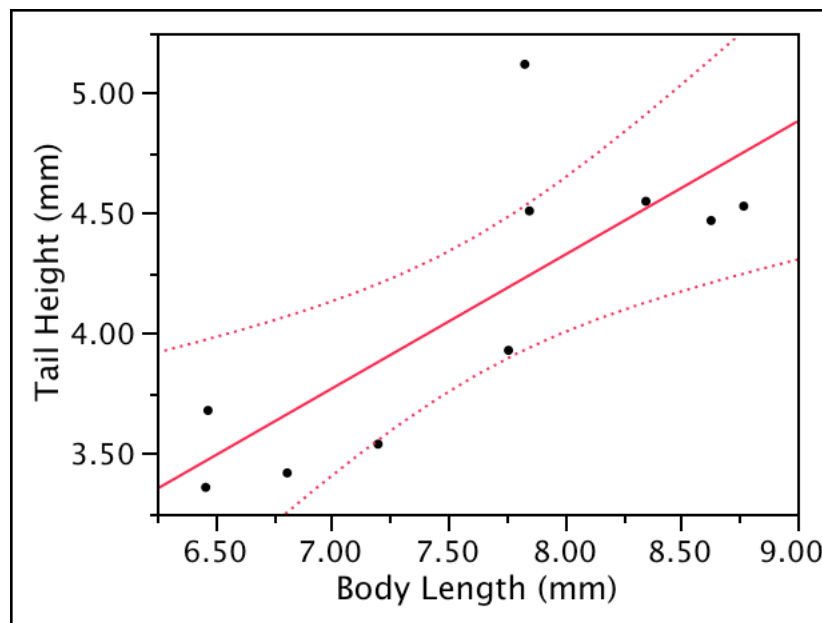


Figure 2. Relationship between body length and tail height for *Bombina orientalis* tadpoles (N=10) with 95% confidence bands for the slope

$$\text{Tail Height (mm)} = -0.12 + 0.56 * \text{Body Length (mm)}$$
$$R^2 = 0.62$$

As body length increases, tail height statistically significantly increases in *B. orientalis* tadpoles (ANOVA, $F=12.8$, $df=1,8$, $P=0.0072$). About 62% of the variation in body length explains the variation in tail height.

Note differences between the original JMP output and the format to hand in:

- No text goes above the figure.
- Figure legend goes below the figure.
- Put linear equation and R^2 below the figure legend.
- Numbers have same number of digits to the right of the decimal place as original measurements.
- Description of pattern and testing of null hypothesis goes last.
- The % of variation explained comes from the R^2 value.
- If your P value was >0.05 , your statement would be more like this:

There is no statistically significant relationship between body length and tail height in *B. orientalis* tadpoles (ANOVA, $F=0.1835$, $df=1,8$, $P=0.6797$).

- Save** files from the **Desktop** to your **Home Server** folder.
- Make sure that both partners' names are on both reports.
- Print to **Ned's Lab** (B5) or **Carey's Lab** (B7) printers.
- Please turn in only one copy of each report with both partners' names at the top.
- Turn in two separate one-page reports (stickleback fish and *Bombina* tadpole) to your lab instructor before you leave lab today.
- Log Out.** (Do not Shut Down.)
- Leave the computer open so that it can get automatic updates overnight.

CONGRATULATIONS!! You have finished your first biology laboratory.

References

- Arrighi, J. 2007. Making sense of unpredictable environments: variable temperature effects on embryonic growth in the Oriental Fire-Bellied toad (*Bombina orientalis*). Reed College Senior Thesis. Reed College. Portland, OR.
- Lencer, E. 2005. Morphological variation and demography of stickleback populations in the Reed College Canyon. Diack Summer Field Research. On file in Brehm Biodiversity Center. Reed College.
- Manoukis, N. 1995. A population study of threespine sticklebacks (*Gasterosteus aculeatus*) in the Reed College Canyon during the summer of 1995. Diack Summer Field Research. On file in the Brehm Biodiversity Center. Reed College.
- Qi, M. 2005. Morphological variation in populations of threespine stickleback (*Gasterosteus aculeatus*) in the Reed College Canyon. Reed College Senior Thesis. Reed College. Portland, OR.