Name _____

ECOLOGY and EVOLUTION
Week 2: November 5 – 9, 2012

Stickleback Fish in the Reed Canyon (Part I)

PLEASE READ the entire lab manual before coming to lab, and be sure to have your Bio Binder and Lab notebook with you.

NOTE: You will be working **with the same partner** this week and next week.

All the new analytical tools you have developed over the past weeks enable us to get into the field and work with the animals in their natural environments. Due to the amount of time to implement studies in the field, we are taking a scaled back approach on the intricacies of the experimental design.

The documents in the Bio Binder: Sections E, F, G, H, I and relevant parts of Section J should be familiar to you at this point. Please continue to work on these and study them during non-laboratory time.

Specifically you will:

1) work with live threespine stickleback, *Gasterosteus aculeatus.*

2) become familiar with our private wild space, the Reed Canyon. Reed College is several years into a major restoration of the canyon, and students have played an extraordinary large role in this endeavor. One goal of the restoration project is to allow the canyon to become a living laboratory for Reed students. The discovery that the Reed Lake harbors a large population of *G. aculeatus*, made the use of the study site a simple choice. Check out the website on the Bio101 home page or click here Reed Canyon (http://web.reed.edu/canyon/). You should pay particular attention to the Natural History, Curriculum, and Restoration links on the Canyon home page. Anyone who is interested in working on this website should see Prof. Kaplan. Anyone interested in working in the Canyon with full time restoration specialist, Zac Perry, should contact him directly (perryz@reed.edu).

3) learn new skills in population census methodology (relative census counts and mark-recapture) and explore the morphological variation among two fish habitats that are isolated from each other.

THE SUNDAY BEFORE LAB 3-4 pm– The 20-40 students who volunteered and signed up last week will set two minnow traps at each numbered site, one in the eastern semi-isolated Ritmanis Pond and the other in the main Reed Lake. Meet at the Physics loading dock.
Wear appropriate clothes for fieldwork for setting traps on Sunday and for your lab day. You may get muddy. You must wear socks in the hipwaders.

ON YOUR LAB DAY - Twenty sampling sites have been staked out around Ritmanis Pond (Fig. 1 and 2) and another 20 around the west end of Reed Lake (Fig. 1 and 3). These sites were selected for ease of access and quality of habitat.

As soon as you get to lab, **sign up with one partner for a specific site # on the sign-up sheet** near the aerial views of the lake and pond on the wall outside of the intro lab B7.

We will do the following together after everyone comes to lab.

- Each pair of students should bring: a) a ruler with your names and Group ID (M1-F24) clearly written on tape and placed on the inch side of the ruler, we only want to see mm, b) two small dip nets, c) two nail clippers, d) your lab notebooks, e) a pen or pencil, and this lab handout.

- On the Physics loading dock outside of the Biology basement West door, take off your shoes (wear socks) and find a pair of hip boots that fit. You will need the boots at the Ritmanis Pond. **Do not go into the Reed Lake.**

- Get **two walking sticks** and **two buckets**. Depart from the North end of the loading dock area behind the Biology building to gravel stairs leading down to the canyon trail. Turn right at the bottom of the stairs. There will be signs leading you to Ritmanis Pond.

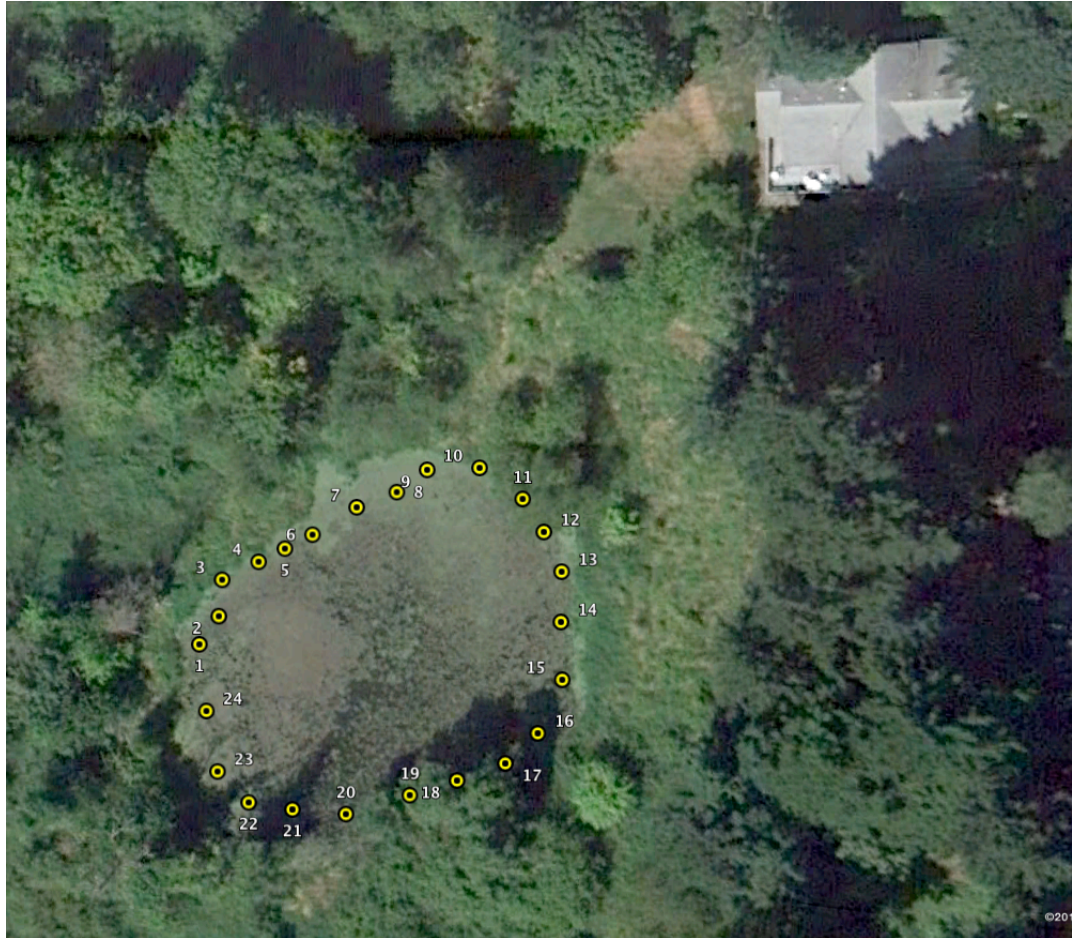Figure 1. View of Reed Lake and Ritmanis Pond to the east (right)

Figure 2. Close up view of Ritmanis Pond (orientation with North at top. Only 20 sites will be studied this year.)

- Follow the signs directing you to Ritmanis Pond. Use the lawn by the house at the north edge as a staging area. You will have to carefully tread around the outer edge of the pond occasionally getting into the water. Use the walking stick to stabilize yourself as you walk in the pond. The map above is a rough estimate of where the sites will be, but please look for the labeled sites once you get to the pond edge.

- PLEASE NOTE: This is a sensitive riparian area, and we have taken great effort to make it easy for you to not tread on plants or destroy the water's edge. Please be extremely careful, especially at the pond edge. LEAVE AS SMALL A FOOTPRINT AS YOU POSSIBLY CAN.

RETRIEVING FISH –

• When you get to your sampling site, fill <u>both</u> buckets about half full of relatively clear pond water.

• Pull in the trap. Before opening the trap, carefully turn the trap on its side and gently knock all the fish to one end. Open the trap and then empty all trapped fish into one of the buckets.

• Reset the trap at your site for tomorrow's lab to collect (unless it is **Friday**). To reset the trap, make sure that the trap is put together properly and that the seams that join the two halves of the trap are tight, then put your hand through the loop provided on the 7 m cord and <u>hold on to the cord</u>. Throw the trap out as far as the line allows. Let the trap settle horizontally on the bottom. If the water is shallow, you only need to have the entrance holes of the traps submerged in order for the trap to work. PLEASE - AVOID SNAGS - PLEASE.

• Fasten the cord to the shoreline with a labeled tent stake.

• If you are in **Friday** lab, instead of resetting the traps, please bring the traps back to the loading dock and wash them off for use again on Sunday.

• Bring the fish in the bucket and your gear up to the photography station on the lawn by the house North of the pond.

  When it is your turn and the photographer is ready for you, place your ruler mm and name side up in the photographic field. Randomly catch **up to 10** fish and place in the photographic field next to the ruler. The photographer will assist you, and a photographic image will be taken of the fish. Later in lab you will have access to the image for measuring with **ImageJ** software.

• With your partner, look at the pelvic spines of <u>each</u> fish in the bucket and note whether they have been clipped or not.

• If yes, record as **previously clipped** in your lab notebook.

• If not, clip one spine 1/2 off and record as **just clipped**.
  o Put all clipped and counted fish into the second bucket. The sum of the **previously clipped** and **just clipped** fish is equal to the **total number** of fish in your trap.
  o Record the numbers in your lab notebook.
  o If your trap has other species, describe what you saw and write it in your lab notebook.

- When done photographing up to 10 fish and counting and clipping <u>all</u> fish, return all fish to the Pond **to the site at which you caught them**.

- Help other people, who may have caught more fish than you, to finish counting and clipping their fish.

- When <u>everyone</u> is done, we will make our way to the **Reed Lake** site.

- At this point, you will have no fish or traps in your possession (unless it is **Friday**)! The traps set at Ritmanis Pond and Reed Lake should be left for the following lab day's students to collect.
- Travel along the South path of the canyon back towards the amphitheater and the land bridge.

At Reed Lake: Do not walk into the Reed Lake.

Collect the second trap at your site #. The map below is a rough estimate of where the sites will be, but please look for the labeled sites once you get to the lake edge.
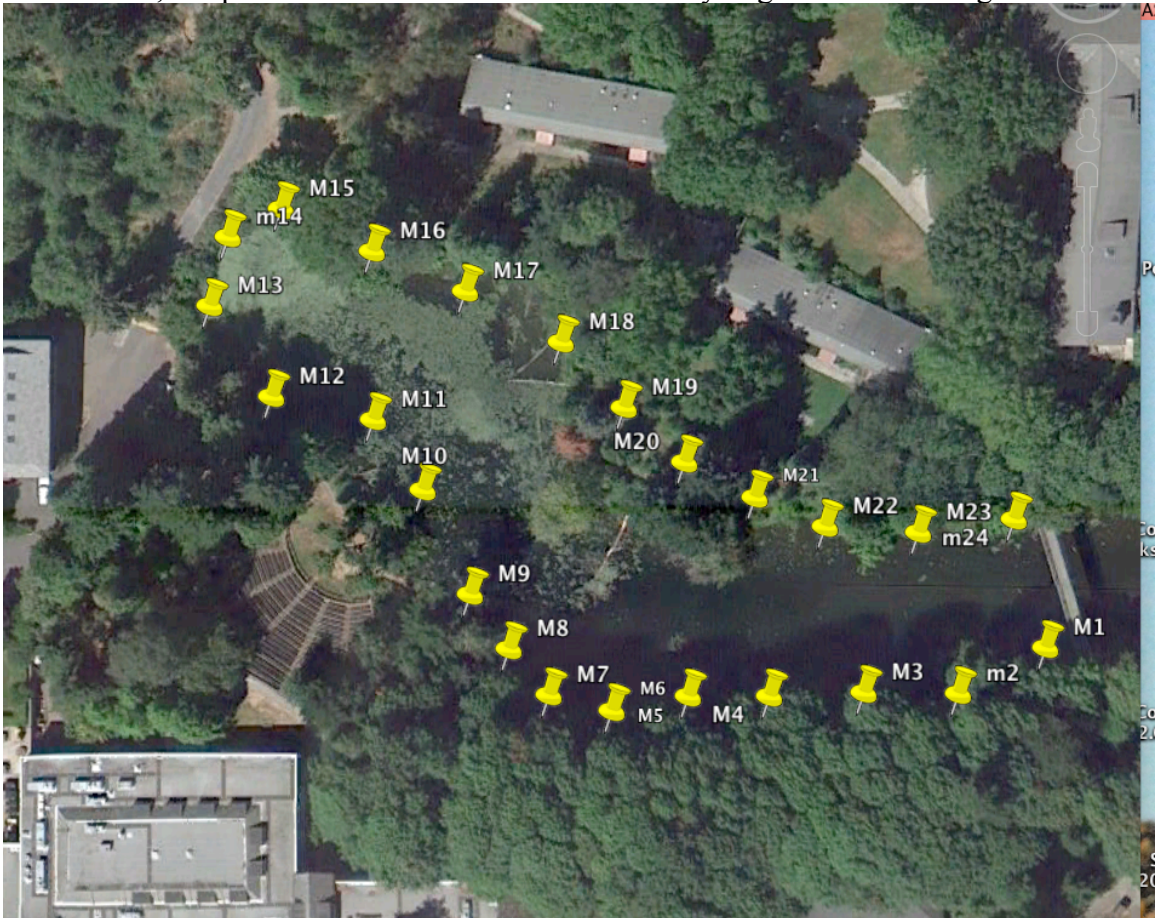

Figure 3. West end of main Reed Lake. (North orientation up. Only 20 sites will be studied this year.)

- As before, fill both buckets half full of lake water. Collect all stickleback in your trap and put them in one of the two buckets. Reset the trap for tomorrow's lab to collect (unless it is **Friday**).

- Bring the fish in the bucket up to the photography station just past the fish ladder on the land bridge. When it is your turn and the photographer is ready for you, place your ruler mm and name side up in the photographic field.

  Randomly catch **up to 10** fish and place in the photographic field next to the ruler. The photographer will assist you, and a photographic image will be taken of the fish. Later in lab you will have access to the image for measuring with **ImageJ** software.

- With your partner, look at the pelvic spines of <u>each</u> fish in the bucket and note whether they have been clipped or not.

- If yes, record as **previously clipped** in your lab notebook.

- If not, clip one spine 1/2 off and record as **just clipped**.
    - Put all clipped and counted fish into the second bucket. The sum of the **previously clipped** and **just clipped** fish is equal to the **total number** of fish in your trap.
    - Record the numbers in your lab notebook.
    - If your trap has other species, describe what you saw and write it in your lab notebook.

- When done photographing up to10 fish and counting and clipping <u>all</u> fish, return all fish to the Lake **to the site at which you caught them**. Collect all your gear.

- Help other people, who may have caught more fish than you, to finish counting and clipping their fish.

- When <u>everyone</u> is done, return to the loading dock. At this point, you have no fish or traps in your possession (unless it is **Friday**).

    - Use the hose to wash off your boots and buckets (and traps if it is **Friday**). Hang pairs of boots together, neatly as you found them, sorted by size. Leave buckets on the loading dock.

    - Return the walking sticks to their container.

- Go to lab. Proceed immediately to the next data storage section and come back to the images when they are available.

- When the two images with your Ritmanis Pond and Reed Lake fish are available, find the images in a file folder on the **Courses Server**.

Biology->Biology101 102 Intro Biology ->3rd Evolutionary Ecology->Kaplan->LABORATORY MATERIALS->Lab 2 Stickleback I->stickleback photos

Drag your images to your desktop before opening.

- Using **ImageJ**, measure the **standard length** and **depth** (see Fig. 2 from week 1) of each of the photographed fish from the Pond and the Lake.
- Record these in your lab notebook with proper labeling.

DATA STORAGE – You and your partner will be responsible for posting both Ritmanis Pond and Reed Lake stickleback fish count numbers (**previously clipped** + **just clipped** = Total) up on the front board. Even if you had 0 fish, you need to record that on the board.

You will also be responsible for storing your size measurement data appropriately on a blank data template as described below.

1) Log on to the **Courses Server** using the **red R** (AFS) in the dock.

2) **Courses Server->Biology ->Bio101.102 Intro. Biology-> 3rd Evolutionary Ecology- Kaplan -> Laboratory Materials-> Lab 2 Stickleback I**

3) Drag the file called **COPYTHISBLANKSTICKLEFILE** to your **Desktop**. Note: Always drag files from the Server to your Desktop before opening the copy on your Desktop. If you open a file directly from the Server, no one else will be able to use that file.

4) Double click on the **Desktop** copy of **COPYTHISBLANKSTICKLEFILE** (the original is locked to avoid any accidental changes) and **Save As**...your **Group ID (M1, T3, W4, Th7, F20 etc**.) to your **Desktop**.

5) Note that the **first row** is an example to show you the format and the normal range of sizes in mm. Some columns have been entered for you.

Starting in row 2, enter in **rows 2-21** your names, Group ID, day of week, Location (Lake/Pond) and sampling site. Every row needs to be filled in as the dataset gets sorted in different ways. **Copy** and **Paste** are useful here.

If you did not find any stickleback at your site, enter 10 rows of all the labeling information for the **Pond** trap and another 10 rows for the **Lake** trap, but leave the Standard Length and Body Height measurement columns empty. Do not enter 0 here. Otherwise, enter the data for **each individual fish** on the **same** row.

Select the example row number **1** and use the **Rows** menu ->**Delete Row**.
Your file should now have 20 rows (10 for the Pond and 10 for the Lake).

Save your completed data set to your **Desktop**. Drag it to your **Home Server** for safety's sake.

Ask your lab instructor to check your file before you put it in the drop box (step 6).

6) **Close** the file first, and use **files.reed.edu** to **Upload** your file (**with your Group ID as the file name**) into the **field data drop box** folder in **Lab 2 Stickleback I** folder on the **Courses Server**.

You can see that your file went into the **field data drop box** folder, but it is permission protected, so you can't change or remove your file once it is in this folder.

After making <u>sure</u> that your dataset is successfully in the data drop box (check with your lab instructor), take a break and come back around 3:45pm when the class dataset will be ready for analysis.

DATA ANALYSIS and REPORTING PRELIMINARY RESULTS

Now, we will use today's dataset (**M, T, W, Th, or F - stickleback size data**) to test two null hypotheses.

Drag the file to your **Desktop**.

Note: Always drag files from the Server to your Desktop before opening the copy on your Desktop. If you open a file directly from the Server, no one else will be able to use that file.

1. Test the null hypothesis that there is no significant difference between the mean size of fish from the Reed Lake vs. Ritmanis Pond.

   Perform a one-way analysis of variance (ANOVA) using **Analyze->Fit Model** in **JMP.** Use either **Standard Length** or **Body Depth** as the **Y** variable and **Location** as the **Construct Model Effects** variable. Don't forget to select **Emphasis: Minimal Report**. Be sure to create an **LS Means Plot**.

   • **Select, Copy** and **Paste** the output into **Word**.
   • From the Courses Server, drag a copy of the ANOVA report template.doc file to your **Desktop** from the **Lab report templates** folder.
   • Use the **ANOVA report template.doc** (same as in week 1) and modify for this lab.

2. Test the null hypothesis that a linear relationship between body length (X) and body height (Y) are not significantly different between Reed Lake and Ritmanis Pond.

   Before we test the hypothesis, select **Fit Model**. This time enter **Body Height** as the **Y variable** then enter **Standard Length** in the **Construct Model Effects** box **(X variable)**. Select **Emphasis: Minimal Report. Run Model.**

   You will see the **Regression** output that you are used to seeing, except this time you got it not by using the **Fit Y by X** option but by using the **Fit Model** option.

   Note that you combined all your data from both lake and pond in this graph. Is there a significant relationship?

3. But, suppose we want to test the hypothesis that there is no significant difference in the lines describing length and height that you could draw for Reed Lake and Ritmanis Pond separately. This is called an **analysis of covariance** (ANCOVA). Perform the ANCOVA (Bio Binder H-15 and H-16).

- **Select**, **Copy** and **Paste** your ANCOVA into a blank **Word** document.
- The ANCOVA table has 3 P-values and is made from a combination of the **ANOVA** Table and the **Effect Tests** Table.
- From the **Courses Server**, drag these <u>two</u> files to your **Desktop** from the **Lab report templates** folder:

    ANCOVA report template.doc

    ANCOVA instructions.doc

- There are three null hypotheses to address and three F, df, and P-values to report. See page I-10 in the Bio Binder for more information.

Modify the **ANCOVA report template.doc** for your analysis.

Use the color-coded **ANCOVA instructions.doc** on screen for help in creating the final table and for final statement options.

We will work on the results of our mark recapture study and relative census counts next week.

Final Instructions:
- Make **PDF** documents from the **Word** documents from step 1 (ANOVA) and step 3 (ANCOVA) above. Keep the ANOVA and ANCOVA reports separate.
- Use the standard filename format.

    M_Abel_Bell_ANOVA.pdf
    and
    M_Abel_Bell_ANCOVA.pdf

- **Upload** to the **Lab Report Drop Box** <u>one</u> copy of each with both partners' full names, lab day and lab instructor at the top as in the template.

- They are due at the beginning of next week's lab, but you can turn them in today if you are done.

- Always **Save** all files from the **Desktop** by dragging them to your **Home Server.**

*Optional: For those of you who feel comfortable, play with JMP a bit more. (Nothing to hand in.) On the same JMP results output, go to the **Response** arrow and select **Factor Profiling** and then select **Profiler**. Follow the instructions above the two graphs and see if you can get a feel for the dynamic nature of the profiler and the interactions that it is trying to show. It is a VERY cool feature of JMP.*

- **Log Out.**

ECOLOGY and EVOLUTION
Week 3: November 12 – 16, 2012

Stickleback Fish and Tadpoles:
Results and Conclusions

VOLUNTEERS THE SUNDAY BEFORE LAB 3-4 pm - SETTING POND AND LAKE TRAPS – Twenty-forty volunteers will need hipwaders (must wear socks), traps, tent stakes, and wading sticks from the loading dock.

ON YOUR LAB DAY - Report to laboratory at your regular time on your regular lab day for directions.

You must wear socks and field clothes.
As a group, we will go out to collect traps and data.

Today's lab is going to bring to a conclusion the two major experimental approaches that you have been working on in this section. These are the: (1) long-term multifactorial tadpole experiment and (2) the comparative experiment of stickleback fish in the Reed Lake and Ritmanis Pond.

PLEASE READ this entire manual (Week 3) before coming to lab. Be sure to bring your Bio Binder and Lab notebook to lab.

- **On your lab day, come to lab to receive instructions**
- Retrieve traps, collect marked and unmarked count data
- Return to lab, enter stickleback count data
- Conclude *Bombina* experiment, collect data, enter data

- Stickleback analysis in class

- *Bombina* analysis is started in class
- Using entire class dataset available Friday 5pm, finish *Bombina* analysis and report with one partner.

- One **pdf** copy of this more formal lab report is due on Friday November 30 by 1:10pm to the **Lab Report Drop Box** on the **Courses Server**.

Sticklebacks

BACKGROUND: You should review prior lab material on sticklebacks, including the suggested links associated with the labs.

In the first lab, you used the univariate analysis of variance to test the null hypothesis that there was no significant difference in shape between lake fish and pond fish from photos taken in the summer of 2005.

In last week's lab, you again tested for the difference between lake and pond fish with respect to size, and you tested for differences in the bivariate relationship between Standard Length and Body Height depending on the location of the fish. This was done with a new technique called analysis of covariance (ANCOVA).

You will finish your stickleback work by estimating population size in the Pond and the Lake and performing a two-way analysis of variance (two-way ANOVA) to test for the differences in fish size between the Pond and the Lake, depending on the day of the week the fish were measured.

Today

- At the Pond, count and record previously marked and unmarked stickleback caught in your trap. Reset the trap (unless it is Friday). Return fish to the site where you caught them.

- At the Lake, count and record previously marked and unmarked stickleback caught in your trap. Reset the trap (unless it is Friday). Return fish to the site where you caught them.

- Once back in lab, you will enter 6 numbers on the board in lab (marked, unmarked, and total number caught for both Pond and Lake).

- We will complete the stickleback analysis after we have completed the *Bombina* data collection.

Bombina tadpoles

BACKGROUND: You have been exposed to some of the biology of this species in Lab I, and you should review the material.

During Lab I, you developed the skills of regression (bivariate) analysis and looked at the relationship between the log of tail height and log body length in ~two week old (14 days post fertilization) *Bombina* tadpoles. You tested the hypothesis that that there was no significant relationship between tail height and body length or more formally, you tested whether you could reject the null hypothesis that the slope of the best fit straight line through the points was significantly different from zero. In addition, you considered whether the slope of the logarithmic line was different from 1.0 and considered allometric scaling of shape with size.

You set up a multifactor, randomized design that was symmetrical. It is essential that you review the structure of the experiment as presented in that lab handout. Briefly, each lab day and room started with approximately 40 tadpoles divided evenly into: (1) Canyon water with Predator Cue, (2) Canyon water without Predator Cue, (3) Lab water with Predator Cue, (4) Lab water without Predator Cue. Thus, with approximately 20 students in a class with 2 tadpoles each and 4 unique treatments, each of the 4 treatments started with a sample size of 10 tadpoles per class. Over the 10 classes (5 days Ned+5 days Carey) there were approximately 100 tadpoles per treatment with a potential total sample size of N=400.

Now, you will analyze the experiment with today's 80 tadpoles, but your final report will require your **analysis of the entire introductory biology class data set** after it is completed on Friday.

Today

- Find a medium sized Petri dish and a ruler.

- Use a spoon to move your tadpole into the medium Petri dish.

- Let the tadpole calm down by not touching it.

- Wick off as much excess water as you can from the medium sized Petri dish with a KimWipe (in the box in your plastic drawer).

- Gently shake the medium sized Petri dish side to side to slide the tadpole into a "lie-flat" body position.

- Move the ruler under the medium sized Petri dish to measure the tadpole under the dissecting scope.

- Make an estimate to the nearest 0.1 mm (it will be rough) of the body length of the tadpole (not including the tail) and maximum tail height of the tadpoles just as you did before**.**

Figure 1. Tadpole body length = solid line, maximum tail height = double arrow line.

- Represent hind limb developmental stage using a number between 26 and 42 as described in Appendix 1. Ask for help, as seeing the legs can be difficult. If your tadpole's body length is >10mm, you should see some hind limb development.

- Be sure to write the two measurements and hind limb observations down in your lab notebook along with other important details (e.g., time, treatment, grid location, etc.). Repeat for your other tadpole. Hold onto your tadpoles until <u>after</u> you check the results with Ned or Carey.

- Report the body length, tail height, and hind limb data you just collected for your tadpoles to the class data set by giving your 6 numbers (3 for each tadpole) to Ned or Carey. They will enter them into the dataset on the same line that contains your tadpole's first body length and tail height from week 1.

- **<u>Only after</u>** Ned or Carey have checked your 6 numbers, place your tadpoles in a communal tank. They will live there for the remainder of the week and then be taken up to the Kaplan laboratory where they will be followed through metamorphosis. Ask Bob to show you the many one-year old survivors from last year's experiment.

- Pour your tadpole's water down the drain, <u>remove the tape labels</u>, rinse, and place the rinsed containers and lids in the dishpan by the back sink.

- Now you are done with *Bombina* data collection and will begin the stickleback analysis. You will analyze *Bombina* data after the stickleback data are analyzed.

Stickleback Analysis

Population size estimate

How many fish are there in the lake? How many fish are there in the pond?

- Drag the file called **markrecapCALCULATO**R from the **Courses Server** to your **Desktop**. When you open the file, notice class and location data for other years. Note there are data for Ritmanis Pond and Reed Lake from other years that can be compared with what we find today.

- We will provide the combined totals for total numbers and total marked for both Ned and Carey's sections for today's analysis. While waiting for the combined totals, use the **markrecapCALCULATO**R with your personal site number data that you got today to estimate population size.

- Answer these questions in your lab notebook while waiting for the class dataset.

1. If you had no fish, what would your estimate be?

2. If you had no marked fish, what would your estimate be?

3. If you had only marked fish, what would your estimate be?

4. If you had some marked and some unmarked fish, what would your estimate be?

- Using the **class, combined totals**, enter the total number of fish caught today and the total number of marked fish caught into appropriate columns in the **markrecapCALCULATOR** at the bottom of the file in the 2012 section.

- Do this for **both** the Pond and the Lake separately.

- **Save** the file to your **Home Server**.

- Answer these questions in your lab notebook.

5. What is the estimate obtained from the whole day of data combined for the pond?

   What is the estimate obtained from the whole day of data combined for the lake?

Stickleback Body Size: Two-Way ANOVA
See Bio Binder JMP Instructions H-17-19 and Statistics Explained I-11.

Did different lab days catch fish of different sizes in the pond and the lake?

> The aim of this exercise is to give you a better feel for the size of the fish in the two bodies of water. The output of this two-way ANOVA will be similar to that used for your full tadpole report to be handed in next week. This week we want to see and comment on this output for the stickleback analysis. You will then be able to use this format and our comments as a guide for your tadpole report.

Drag **SB week 1 size data** from the **Courses Server** to your **Desktop**.

The two-way model that you will run looks like the following:
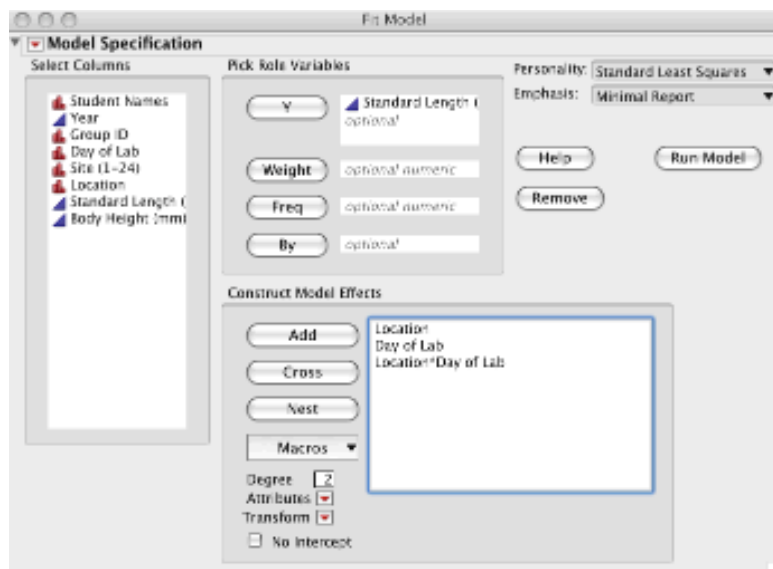
Response Standard Length (mm)
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	4173.905	596.272	7.3235
Error	1001	81500.441	81.419	Prob > F
C. Total	1008	85674.346		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Location	1	1	2731.0346	33.5430	<.0001*
Day of Lab	3	3	378.2382	1.5485	0.2004
Location*Day of Lab	3	3	651.3302	2.6666	0.0466*

► Effect Details

Instead of using the LS Means Plot as we did with the One-Way ANOVA, use the **Chart** option under the **Graph** menu to show standard error bars around the means and to better communicate what's happening in the study.

See Bio Binder JMP Instructions H-20-21.

After selecting **OK**, select the red arrow next to **Chart** -> **Add Error Bars to Mean** -> **Confidence Interval**.

Select, Copy and **Paste** into a blank **Word** document.

The output below is a combination of information from the **ANOVA** and **Effects Tests** tables similar to what you did last week for ANCOVA.

Use the **ANCOVA instructions.doc** file as a guide again.

Use the table in the **Two Way ANOVA template.doc** file and type in your numbers.

Save your file to your **Desktop** and drag to your **Home Server**.

Print one copy of this file and have Carey, Ned, or Bob comment on it.

Save it and the comments in your Bio Binders as a model for your tadpole report.

The Figure and Table below are examples of acceptable output for both this stickleback exercise and the tadpole report Results section.

Your stickleback output and tadpole report will be unique to your dataset and analysis.

You should change the Y-axis range (double click on the Y-axis numbers) to accentuate the differences.
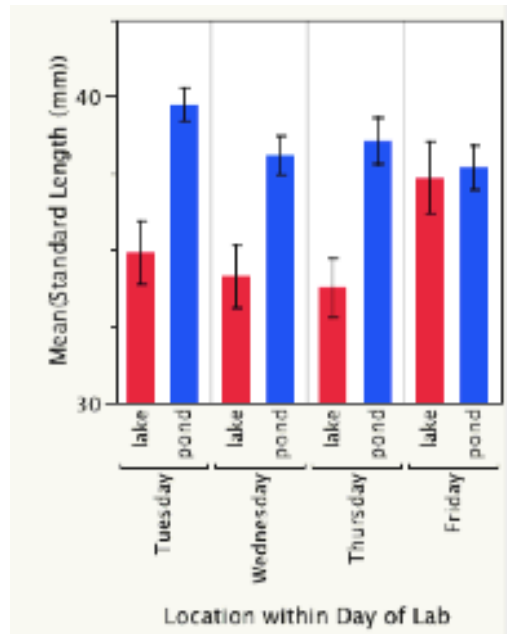
Results

Figure 1. Mean standard length (+/- 95% CI) of stickleback fish (*Gasterosteus aculeatus*) in Ritmanis Pond and Reed Lake by day during the week of September 11, 2007 (N=1009).

Table 1. Analysis of Variance for standard length of stickleback fish[a] as influenced by location, day of week, and the interaction of location with day of week.

Source	DF	Sum of Squares	Mean Square	F Ratio	P Value
Location[b]	1	2731.0	2731.0	33.5	<0.0001
Day of Lab	3	378.2	126.1	1.5	0.2004
Location*Day of Lab	3	651.3	217.1	2.7	0.0466
Error	1001	81500.4	81.4		
Total	1008	85260.9			

[a] *Gasterosteus aculeatus*
[b] in Reed Lake vs. Ritmanis Pond

The mean length for pond stickleback fish (38.5 mm) was statistically significantly greater than the mean length for lake stickleback fish (35.0 mm) (ANOVA, F=33.5, df=1,1001, P<0.0001).

There is no statistically significant difference over the 4 lab days in the mean Standard Length of stickleback fish (ANOVA, F=1.5, df=3,1001, P=0.2004).

There is a statistically significant interaction effect between Location and Lab Day for Standard Length of stickleback fish in that there was less difference between Reed Lake and Ritmanis Pond lengths on Friday as compared to the other 3 days (ANOVA, F=2.7, df=3,1001, P=0.0466).

As an optional analysis for those of you who'd like to pursue the allometric relationships that we looked at earlier -
Use the entire class data set for stickleback in the two locations.

Transform length and depth to logarithms.

Run an analysis of covariance on the two logarithmic regressions.

Answer the following questions:
(1) Do either the pond or lake stickleback demonstrate some sort of allometry?

(2) If so, are the allometric relationships different?

Bombina Analysis

- You will be conducting preliminary data analysis and exploration of today's 80 tadpoles with a partner.

- At the time of your lab, the data set is still incomplete. Get today's file from the **Courses Server**. Because each day is a complete experiment in itself (each lab is a spatial block with two sub-blocks for lots of variance introduction), the available data are complete enough for you to perform preliminary analyses. This data exploration is a vital part of performing the final analysis on the entire data set. The entire data set (**Tadpole class data**) will be available Friday at 5 pm on the **Courses Server**. Drag to your desktop before opening.

- **Advice -** As you begin your analysis, present your data as a two-way analysis of variance of one response variable. Remember the questions: (1) Does Canyon water vs. Lab water influence body size or shape? (2) Does the predator cue vs. no predator cue influence body size or shape? and (3) Does the predator cue cause an interaction with water type? Another way to state the interaction effect is: Were two potential stressors greater than the sum of each separately?

- Two – way ANOVA is discussed in the Statistics Explained section of the Bio Binder page I-11 and in the stickleback analysis section above. We are deliberately not giving you specific instructions now. You need to understand what you have learned about analyzing data and articulating hypotheses.

- Take a look at the possible response variables. Substitute them as Y - variables and look for patterns of interest to you, which you could decide to use for your final report. Are there variables that you can think of that you might like to construct that are not in the table? Ask your instructors for help using Formula in JMP.

- For your final report, you should commit to analyzing just one Y variable that constitutes one column of numbers. Avoid ANCOVA and allometry.

Instructions for your Final Tadpole Report to be done with a partner

A template file called **tadpole report template.doc** is available on the **Courses Server** to get you started with the format requested.
It uses double line spacing, 1-inch margins, and Times 12 pt font.

Introduction (no more than one-half page). Materials and Methods (no more than one-half page). Results (no more than one page Two Way ANOVA template and one-half page of summary statements). Discussion (no more than one-half page). References (2 to 4 journal articles).

Bio Binder sections S, T and U are useful, but not exactly in the form requested above. **Note:** for this report you will <u>not</u> need an Abstract.

- Introduction: Focus your thesis in biological terms. Why is it an interesting problem to you? Which of the concepts associated with this experiment will you focus on (e.g., plasticity, pollution, predators)? What interests you about this study? Cite at least one reference within the introductory paragraph in the following form at the end of the sentence in which you use it (e.g., Smith, 1993).

- Materials and Methods: In this section write what materials you used, how the data were obtained, and how the experiment was structured in order to test particular hypotheses. What software did you use to do the analyses? See Bio Binder page S-3.

- Results: Use the **Two Way ANOVA template.doc** for this section.

- Discussion: Here is where you discuss the meaning of your results in terms of your own interpretation of the graph and table and also in terms of how it relates to the original thesis that you raised in your introductory paragraph. Cite at least one additional reference here. What you conclude should be the final few sentences of your discussion.

- References: Examples are provided below. No Internet links or Wikipedia citations are acceptable. Use only the standard form that you see in the examples below.

Highly recommended articles are on the Courses Server and/or linked from the class website. http://academic.reed.edu/biology/courses/BIO101
Detailed Syllabus->Bio101-Fall->Dr. Kaplan:Lab->Lab III

Some advice about the primary literature:

The literature on the topic of our tadpole experiment is very large and could easily take several months to evaluate. Pursue some of the following references at your discretion. Several are in pdf form on the Courses Server for your convenience but you should attempt to use the on-line e-journal searches and search engines that the library website offers. Use the Database Web of Science. Search for topics: tadpole, predator, development. These are invaluable tools to access the primary literature.
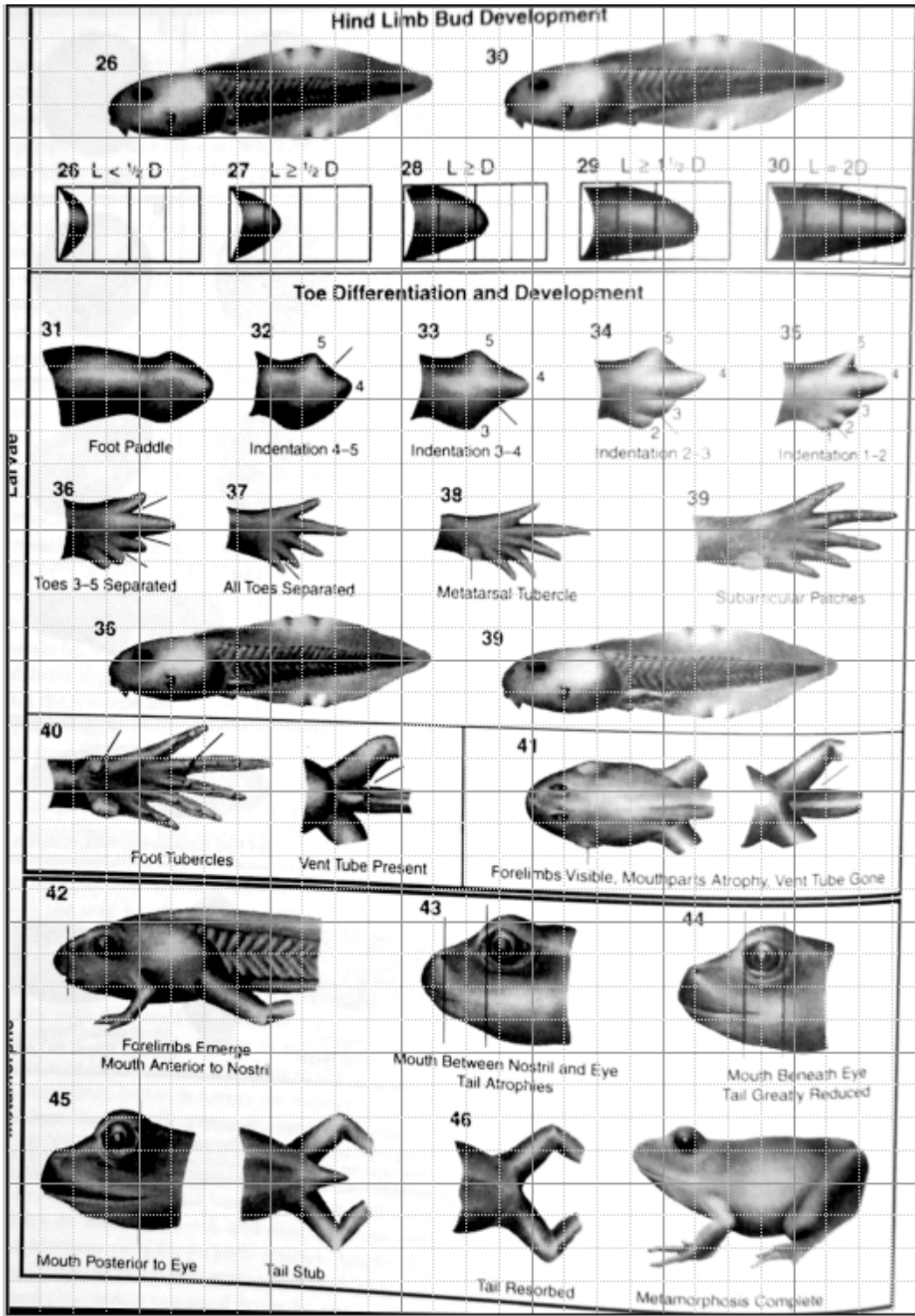
For this report, cite one paper that deals with each of the hypotheses either implicitly or explicitly. As a suggestion site Earl and Whiteman, 2010 for the canyon water hypothesis, or Ortiz-Santaliestra *et al.* 2011 and Sih *et al.*, 2004 for a general approach to interactions and their importance (i.e., does predator cue water result depend on water source? Does water source response depend on predator cue absence or presence?)

> One pdf report for two partners is due on Friday November 30 by 1:10pm. Use the standard filename format. M_Able_Bell_tadpole.pdf

> **Upload** using **files.reed.edu** to the **Lab Report Drop Box** on the **Courses Server**.

References

Earl, J.E. and H.H. Whiteman, 2010. Evaluation of phosphate toxicity in Cope's gray treefrog (<u>Hyla chrysoscelis</u>) tadpoles. Journal of Herpetology 44:201-208.

Hoverman, J. T. and R. A. Relyea. 2007. How flexible is phenotypic plasticity? Developmental windows for the induction and reversal of inducible defenses. Ecology 88:693-705.

Kaplan, R. H. and P. C. Phillips, 2006. Ecological and developmental context of natural selection: maternal effects and thermally induced plasticity in the frog *Bombina orientalis*. Evolution 60:142-156.

Ortiz-Santaliestra, M.E., Fernandez-Beneitez, M.J., Lizana, M. and A. Marco, 2011. Responses of toad tadpoles to ammonium nitrate fertilizer and predatory stress: differences between populations on a local scale. Environmental Toxicology and Chemistry 30:1440-1446.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology and Systematics 37:637-669.

Sih, A., A. M. Bell and .J. L. Kerby. 2004. Two stressors are far deadlier than one. Trends in Ecology and Evolution 19:274-276.

Hind Limb Bud Development

26 · 30

26 L < ½ D · 27 L ≥ ½ D · 28 L ≥ D · 29 L ≥ 1½ D · 30 L = 2D

Toe Differentiation and Development

31 — Foot Paddle
32 — Indentation 4–5
33 — Indentation 3–4
34 — Indentation 2–3
35 — Indentation 1–2

36 — Toes 3–5 Separated
37 — All Toes Separated
38 — Metatarsal Tubercle
39 — Subarticular Patches

38 · 39

40 — Foot Tubercles · Vent Tube Present
41 — Forelimbs Visible, Mouthparts Atrophy, Vent Tube Gone

42 — Forelimbs Emerge, Mouth Anterior to Nostril
43 — Mouth Between Nostril and Eye, Tail Atrophies
44 — Mouth Beneath Eye, Tail Greatly Reduced

45 — Mouth Posterior to Eye · Tail Stub
46 — Tail Resorbed · Metamorphosis Complete

Appendix 1.
Staging series for tadpole limb development. Choose the number that corresponds to the shape of the hind limb. If the <u>front</u> limbs have popped out, choose 42.