

# ECOLOGY and EVOLUTION

Week 4: September 19 – September 23, 2011

## Stickleback Fish and Tadpoles: Results and Conclusions

**THE DAY BEFORE LAB - SETTING 1 POND TRAP and 1 LAKE TRAP** - As close to 4:00 PM as possible (not much before 3:00 PM or after 6:00 PM is OK) on the day BEFORE your regular lab day, meet with your partner and **set one trap at Ritmanis Pond and one trap at Reed Lake at the same sites as last week.** Contact your partner ahead of time and agree on a meeting time and location. You will need hipwaders (wear socks), 2 traps, 2 tent stakes, and 2 wading sticks from the loading dock.

Report to laboratory at your regular time the following day for directions. Wear socks and field clothes. As a group, we will go out to collect traps and data.

Welcome to your final introductory biology laboratory in population ecology and evolution. Today's lab is going to bring to a conclusion the two major experimental approaches that we have been working on. 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 4) before coming to lab. Be sure to bring your Bio Binder and Lab notebook to lab.

- With your partner, set one Pond trap and one Lake trap the afternoon before your lab day at the same site you signed up for last week
- **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 copy of this more formal lab report is due on Friday September 30th by 1:10pm to either Ned or Carey in lab.

## 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 size 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 sophisticated analysis of covariance (ANCOVA). If you have the inclination, you may repeat these analyses for the entire class dataset combined and compare them to your analyses for your lab day.

We will finish our stickleback work by estimating population size in the Pond and the Lake and performing a two-way analysis of variance 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. Return fish to the site where you caught them.
- At the Lake, count and record previously marked and unmarked stickleback caught in your trap. 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 that lab, you developed the skills of regression (bivariate) analysis and looked at the relationship between tail height and body length in ~two week old (14 days post fertilization) *Bombina* tadpoles. You tested the hypothesis 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.

During your second week, 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 48 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 24 students in a class with 2 tadpoles each and 4 unique treatments, each of the 4 treatments started with a sample size of 12 tadpoles per class. Over the 10 classes (5 days Ned+5 days Carey) there were approximately 120 tadpoles per treatment with a potential total sample size of  $N=480$ .

Today you will analyze the experiment with today's 96 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 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 Petri dish with a KimWipe (in the box in your plastic drawer).
- Gently shake the medium Petri dish side to side to slide the tadpole into a “lie-flat” body position.
- Move the ruler under the medium Petri dish to measure under the dissecting scope.

- Make an estimate to the nearest 0.1 mm (it will be rough) of the body length (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.). Hold onto your tadpoles until after 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 2.
- Once Ned or Carey have checked your 6 numbers, **place your tadpole 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.
- Pour your tadpole's water down the drain, remove the tape labels, rinse, and place the rinsed container in the dishpan by the back sink.
- Now we 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 **markrecapCALCULATOR** 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 **markrecapCALCULATOR** with your personal site number data that you got today to estimate population size.
  - Answer these thought 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 2011 section.
  - Do this for **both** the Pond and the Lake separately.
  - **Save** the file to your **Home Server**.
  - Answer this question 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 G-16-17 and Statistics Explained H-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:

Fit Model

Model Specification

Select Columns: Student Names, Year, Group ID, Day of Lab, Site (1-24), Location, Standard Length (mm), Body Height (mm)

Pick Role Variables: Y: Standard Length (mm) (optional), Weight: (optional numeric), Freq: (optional numeric), By: (optional)

Personality: Standard Least Squares, Emphasis: Minimal Report

Buttons: Help, Run Model, Remove

Construct Model Effects: Add, Cross, Nest, Macros

Location, Day of Lab, Location\*Day of Lab

Degree: 2, Attributes: [checked], Transform: [checked], No Intercept: [unchecked]

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*

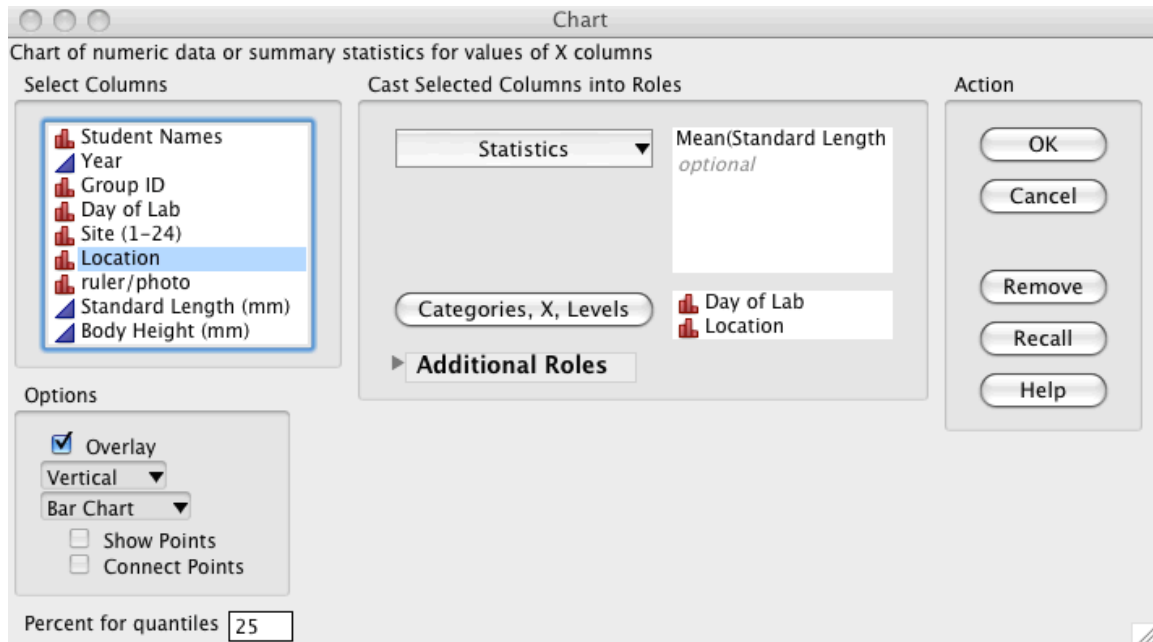
Effect Tests

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 on JMP Instructions G-18 and G-19 and below.



After selecting **OK**, select the red arrow next to **Chart** -> **Y Options** -> **Std Error Bars**.

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

The table 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** two copies of this file (one for each partner) and have Carey, Ned, or Bob comment on them.

Save them 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

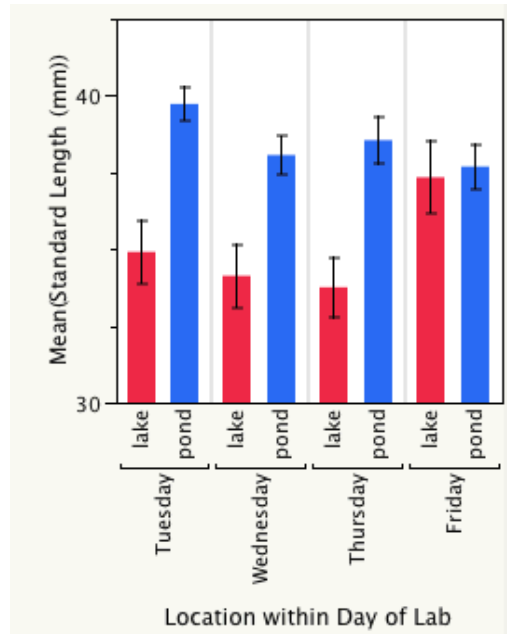


Figure 1. Mean standard length (+/- SE) of stickleback fish (*Gasterosteus aculeatus*) in Ritmanis Pond and Reed Lake by day during the week of September 11, 2007 (N=1009).

Table 1. Analysis of Variance for standard length of stickleback fish<sup>a</sup> 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 <sup>b</sup>	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			

<sup>a</sup> *Gasterosteus aculeatus*

<sup>b</sup> 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 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,  $F=2.7$ ,  $df=3,1001$ ,  $P=0.0466$ ).



## ***Bombina* Analysis**

- You will be conducting preliminary data analysis and exploration of today's 96 tadpoles.
- At the time of your lab (except Friday), the data set is still incomplete. Get today's file from the **Courses Server**. Because each day is a complete experiment in itself (a block), 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**.
- **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 stressors greater than the sum of each separately?
- Two – way ANOVA is discussed in the Statistics Explained section of the Bio Binder page H-11 and in the stickleback analysis section above. We are deliberately not giving you specific instructions now. You need to understand what it is that 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.

## **Instructions for your Final Tadpole Report**

A template file called **tadpole report template.doc** is available on the **Courses Server** to get you started with the format requested. It uses 1.5 line spacing, 1 inch margins, and Times 12 pt font.

Introduction (no more than 1/2 page), Materials and Methods (no more than 1/2 page), Results (no more than 1 page Two Way ANOVA template), Discussion (no more than 1/2 page), References (2 to 4 journal articles)

Bio Binder sections R, S and T are also useful, but not exactly in the form requested above.

**Note:** for this report you will not 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 R-2.
- 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: Some examples are provided below. No Internet links or Wikipedia citations are acceptable. Use only the standard form that you see in the examples below.

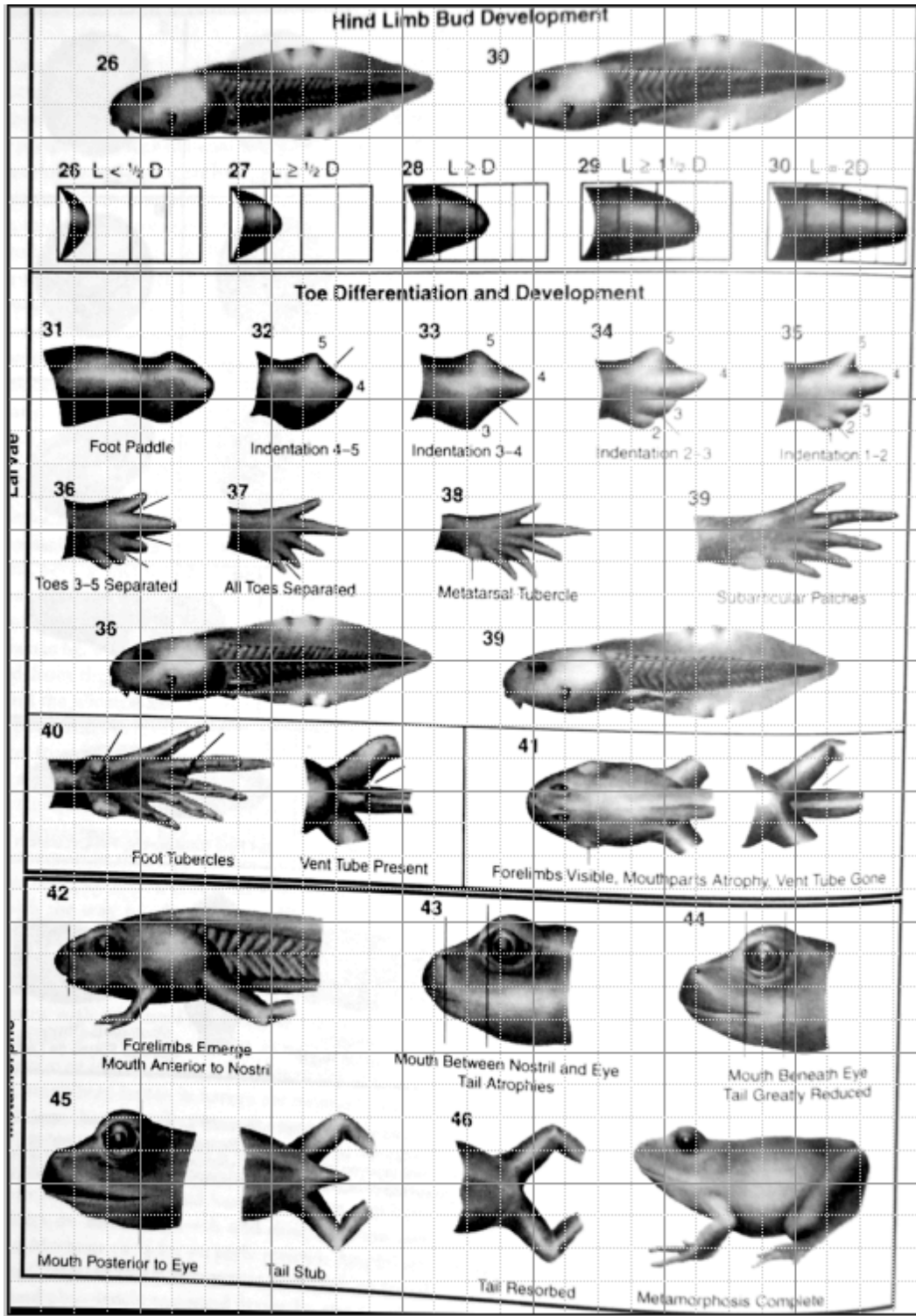
Highly recommended articles are on the Courses Server and/or linked from the class website. <http://academic.reed.edu/biology/courses/BIO101>  
Detailed Syllabus->Bio101-Fall->Dr. Kaplan:Lab->Lab IV

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. Please 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. You'll be amazed at what comes up. 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, perhaps 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?)

## References

- Earl, J.E. and H.H. Whiteman, 2010. Evaluation of phosphate toxicity in Cope's gray treefrog (*Hyla chrysoscelis*) 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.



Appendix 1.

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