# Experimental Design for a population monitoring study: *Hyla regilla* larvae in a high-elevation meadow pond.

Cristi Baldino, Bob Breen, Stephanie Buss, Karen Feldheim, and Jeff Rutter

**Abstract**

Before establishing a long-term *H. regilla* tadpole monitoring program, we conducted a pilot study to determine the optimum sample size and the best methods for sampling tadpoles. We used a stratified sampling technique to estimate the population size of *H. regilla* tadpoles at an ephemeral pond located in Beartrap meadows within the Plumas National Forest. Our estimate of population size and variability suggested that 34 samples would be necessary to detect a 50% decline in the tadpole population. The future study design should include sampling water depth, temperature, pH, air temperature, amount of rainfall to date, and the size of the pond at the time of sampling. Care should also be taken to spend as little time as possible standing in the pond and employ techniques that reduce the chance for pond and sediment disturbance.

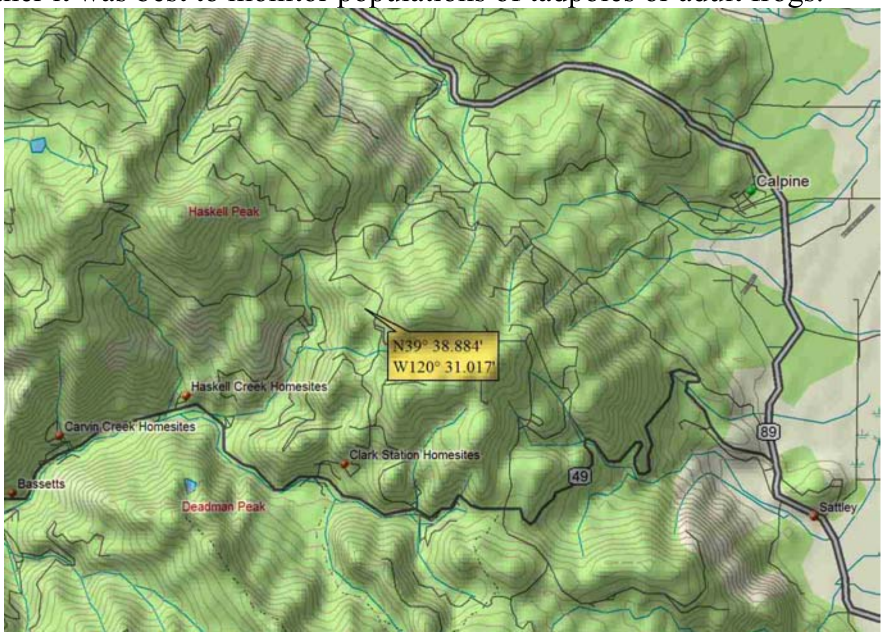
**Introduction**

The pacific tree frog, *Hyla regilla,* is widely distributed throughout western North America from British Columbia to Southern California, Nevada and Arizona from sea-level, brackish estuaries to mountain meadows at 3570 meters (Huybrechts 2001).

Tadpoles are about 10mm at hatching and grow to an adult size of from 25 to 50mm (Gaudin 1965). Eggs are laid in early March at lower elevations and in May at higher elevations (Stebbins 1972). As tadpoles, *H. regilla* feeds on plant material, changing to insects as they metamorphose to adults. Snakes are major predator on tadpoles and adults (DeVito *et al*. 1999). Marco *et al*. (1999) found that when nitrate or nitrite ions were added to water *H. regilla* larvae showed reduced feeding activity, swam less vigorously, showed disequilibrium and eventually died as the concentrations of these ions increased.

On June 26, 2003, we conducted a preliminary study of *H. regilla* tadpole populations at an ephemeral pond located in Beartrap meadows, in the Plumas National Forest at 2167 meters (39, 38' 53"N) and (120 31' 01" W). During our survey the pond covered 1329 square meters.

Our objectives were 1) to collect preliminary data to establish a long-term tadpole population trend monitoring program of *H. regilla* with the ultimate goal to direct the future management of Beartrap meadows as it pertains to livestock grazing, 2) to determine the optimum sample size, 3) to determine the best methods for sampling *H. regilla* tadpoles, and to 4) determine whether it was best to monitor populations of tadpoles or adult frogs.




**Measurement Methods**

Square quadrats, 0.5 m on each edge, were used to collect preliminary data on the Pacific Tree frog tadpoles in a pond located in Beartrap Meadow. The pond was divided into two zones, shallow water and deep water. Shallow water was defined as any section of the pond containing emergent vegetation. Deep water contained no emergent vegetation (although it did contain submerged vegetation). Sample tadpole counts were taken, 5 each from the two zones.

*Tadpole Counts*

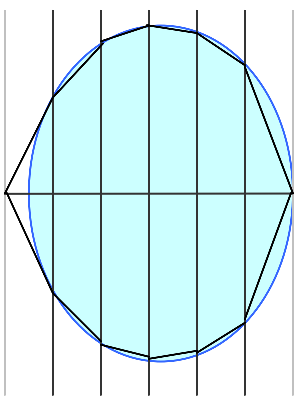
Five 0.5m x 0.5m quadrats were placed in the deep water of the pond. The first quadrat was selected by tossing a stick into the pond. Using a quadrat skeleton made of PVC piping, the quadrat was marked by placing the skeleton into the water and staking the corners of the skeleton. The following four quadrats were then selected by tossing the skeleton to another portion of the deep water zone and where it landed was where the quadrat was marked.

Observers waited ten minutes after the last quadrat was set before counting the tadpoles. Each observer noted the tadpoles were attracted to their legs thus they felt this could have biased the count. A second count was obtained. The observers left the pond and re-entered after ten minutes. Each observer walked slowly into the pond to prevent disturbance and immediately counted the tadpoles once they reached the quadrat. The depth of each quadrat was measured by allowing a pole marked in 5cm increments to rest on the pond's floor at the center of the quadrat.

Five $0.25m^2$ quadrats were placed in the shallow area of the pond using various methods. These methods included blindly picking a spot on the map of the pond that was drawn, throwing a stick into the pond, and just walking up to an area of the pond. Quadrats were again made using a quadrat skeleton and placing stakes at each corner of the skeleton. Once the last quadrat was set observers positioned themselves at least 5 meters from the quadrat and waited ten minutes before they returned and counted the tadpoles. Depth of the quadrat in the shallow zone of the pond was measured using a pole marked in 5 cm increments.

*Pond Area Measurements*

The overall surface area of the pond was measured using four people and two tape measures. The first tape measure was stretched across the pond's narrow axis (width-wise). One person held each end and the tape was stretched taut with each person standing at the edge of water deemed accessible by tadpoles (any visible water connected to the pond). A second pair of people measured off 5 meters perpendicular to the first tape measure (length-wise, along the pond's long axis). The first pair moved along these 5 meters and then repeated the measurement of the width of the pond. This process continued for the entire length of the pond. The overall surface of the pond was then estimated using a sequence of trapezoids, with the estimates for the length-wise ends being approximated by triangles.



This is an example of how the area was approximated. The blue oval represents a region being estimated. The vertical black lines indicate places where measurements were taken. The vertical gray lines indicate places where measurements were not taken, but assumed to be zero for purposes of area estimation. The angled lines form the tops & bottoms of the trapezoids used for estimation. The area of a trapezoid is $\text{area} = \left(\frac{\text{length of left side} + \text{length of right side}}{2}\right)(\text{width})$. The area of a triangle formed at one of the gray lines is the same as a trapezoid where one side has length zero.

The same measurement technique was applied to the "deep" part of the pond. Measurements were taken width-wise within the region of the pond lacking emergent vegetation every 5 meters roughly along the long axis of the pond. The lengthwise points of measurement were not matched with points used for the overall pond area measurement. As in the previous step, the deep pond area was estimated using a sequence of trapezoids and triangles. The area of the shallow zone of the pond (that area containing emergent vegetation) was estimated by subtracting the area of the deep region from the overall area of the pond.

*Other Pond Measurements*

The pH was tested using a 4 way colorimeter pool test kit manufactured by NEP/JED Industries of Scranton PA, with a 6.8 to 8.2 range.

*Population Estimate using Stratified Samples*

As performed, the sampling used a stratified technique—the pond was split into two strata which were sampled independently. Pooling these estimates together requires some special formulas for stratified sampling:

First, we define:

$N_D, N_S$ = number of quadrats in Deep/Shallow zone (square meters/4)

$N = N_D + N_S$ = number of quadrats in pond overall

$n_D, n_S$ = number of quadrat samples taken from Deep/Shallow zone (square meters/4)

$\overline{T}_D, \overline{T}_S$ = mean number of tadpoles counted in Deep/Shallow zone

Then,

$$\overline{T} = \text{mean tadpoles per quadrat in pond} = \frac{N_D}{N}\overline{T}_D + \frac{N_S}{N}\overline{T}_S$$

This is a weighted average, with weights corresponding to proportion of the surface area of the pond.

and

$s_{\overline{T}}$ = standard deviation of the overall mean

$$= \sqrt{\left[\left(\frac{N_D}{N}\right)^2 (s_D)^2 \left(\frac{N_D - n_D}{N_D}\right) + \left(\frac{N_S}{N}\right)^2 (s_S)^2 \left(\frac{N_S - n_S}{N_S}\right)\right]}$$

The Nx/N term corresponds to the proportional weight. The $(N_x\text{-}n_x)/N_x$ term is a correction term for a finite population. Since the number of samples taken in our case is quite small compared to the surface area, these terms are very close to one.

The optimal allocation of samples between the two is determined by minimizing the *standard error* of the overall mean, taking into account the cost of sampling from the two strata. The optimal proportions are:

$$w_x = \text{weight on number of deep/shallow samples} = \frac{N_x s_x}{\sqrt{\text{cost}_x}},$$

where $\text{cost}_x$ is the cost of sampling in the Deep/Shallow zone (x is one of D or S)

$$p_x = \text{proportion in deep/shallow} = \frac{w_x}{w_D + w_S}$$

## Results

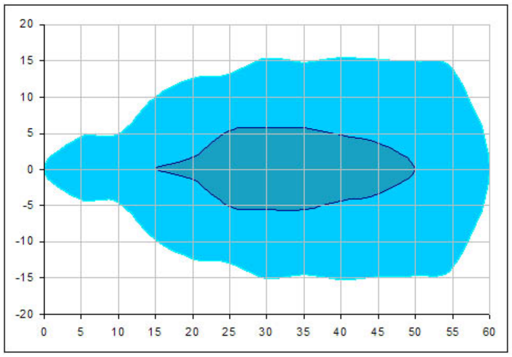
*Tadpole Count*

The average count of tadpoles within each $0.25m^2$ quadrat in the shallow region was 31.0 (standard deviation, $s_S = 30.8$). The average count of tadpoles within each quadrat in the deep zone was 30.0 ($s_D = 19.8$). Using analysis for stratified sampling, the average count per quadrat was 30.8 ($s_{\overline{T}} = 25.2$). The total number of tadpoles in the study pond was roughly estimated using these statistics as 162,138.

*Pond Area*

Overall pond area was estimated at $1{,}329m^2$. Deep pond area was estimated at $259m^2$. Shallow pond area was deduced to be $1070m^2$.

**Figure 1--diagram of pond, with forced symmetry and approximate placement of deep zone.**




*Pond pH*

The pH of both the primary and test ponds was 6.8, slightly acidic.

*Summary*

The overall variability within the pond sampling deep and shallow strata ( $s_{\bar{T}} = 25.2$ ) suggests that 34 samples would be sufficient to detect a 50% decline in tadpole population. The best allocation for these samples is 29 from the shallow and 5 from the deep zone.

| Region | Mean count per quadrat | SD | Area ($m^2$) | Area (%) | Estimated # Tadpoles | Required Sample Size (power=80%) |
|---|---|---|---|---|---|---|
| Overall | 30.8 | 25.2 | 1,329 | **100.0%** | 163,733 | 34 |
| *Shallow* | 31.0 | 30.8 | 1,070 | **80.5%** | 132,680 | *29* |
| *Deep* | 30.0 | 19.8 | 259 | **19.5%** | 31,080 | *5* |

## <u>Discussion</u>

Based on our preliminary data with $\alpha = 0.05$ and $\beta = 0.2$ (power of 0.8), a sample size of 34 (29 samples from the area of emergent vegetation and 5 from the area of submerged or no vegetation) would be necessary to detect a decline of 50% in the number of *H. regilla* larvae in the large pond in Bear Trap Meadows (Dupont and Plummer 1997). Despite the probable variability in tadpole population from year to year due to differences in rainfall and temperature, a decline of this size would be cause for concern.

Although there were noncontiguous areas of standing water containing *H. regilla* larvae throughout the meadow, we chose to concentrate on the largest and deepest pond as the source of the longest-lived habitat for these tadpoles. The meadow site had been visited by several of the observers on June 22, prior to rains on June 23. They reported a definite increase in the number of standing water bodies throughout the meadow on June 26. Investigations on adult *H. regilla* during approximately the same time period in July 2000 and 2001, encountered extremely different habitat conditions. By July 9, 2001, even the large pond was dry. The literature suggests that *H. regilla* choose ephemeral wetlands intentionally to avoid predatory fish and amphibians, such as bullfrogs (*Rana catesbeiana*), which require permanent bodies of water (Huybrechts 2001, Province of British Columbia 2001, Hollingsworth and Roberts. 2000). However, given that their larvae take approximately 2 – 2.5 months to metamorphose, ephemeral pools that dry up before mid-summer would have a low rate of successful metamorphosis

(Gaudin 1965, Nussbaum *et al*. 1983, Province of British Columbia 2001). Cattle grazing in Bear Trap Meadows prior to metamorphosis could also have a detrimental effect on the *H. regilla* population due to trampling of habitat, including algae and diatoms – the main food items of *H. regilla* larvae.

In our surveyed pond in Bear Trap Meadows, *H. regilla* tadpoles appeared, at first observation, to be concentrated in the shallow water area of emergent vegetation. This would support the literature which suggests that *H. regilla* prefer warm water to increase metabolism and accelerate metamorphosis (Goin *et al*. 1978, Province of British Columbia 2001). Nevertheless, the mean number of tadpoles we counted in the area of emergent vegetation and in the area of deeper water with submerged or no vegetation were similar with variability greatest in the former.

The method we chose to sample *H. regilla* larvae was decided only after testing various techniques in a smaller adjacent pond, as well as some trial and error in the larger pond (e.g., discovering that tadpoles are attracted to observers' legs if observers remain in the water). At this time it is not clear how much we dispersed the tadpoles or impacted their spatial distribution when we waded through the shallow area of emergent vegetation to stake the quadrats in the deeper central section, and when we returned later to count the tadpoles. If the tadpoles do return to their original location after the initial disturbance, how soon does this occur?

Another problem that became evident when we entered the pond to stake quadrats, especially in the deeper area, was that sediment kicked up into the water column made it impossible to see tadpoles. Wind-created current then carried the sediment into the area of at least one quadrat. In the future, care should be taken to approach quadrats in a course that will not carry silt toward any of the quadrats, if possible. We also noticed that sediment in the deepest area took longer to settle; possibly due to thicker deposition and/or greater disturbance when placing the quadrat stakes. We suggest a longer waiting period to reenter the water so that sediment disturbed by wading and staking the quadrats has a chance to settle before a count is attempted. (Or perhaps a quadrat marker could be created which could be deployed quickly and with minimal sediment disturbance so that counting could be done without any delay at all.)

Future monitoring should also include water temperature taken in both areas of the pond, pH, air temperature, amount of rainfall to date, and the size of the pond at the time of sampling. We found no invertebrates that could be detected with a 30 x microscope in a soil sample taken from our test pond. Since food supply and predation would have an impact on tadpole success, we recommend that the monitored pond be surveyed for micro- and macroinvertebrates, and algae. Aquatic insects (possibly water boatmen, Family Corixidae) were plentiful in the large pond, but their impact on the tadpole population either through predation or competition is not known at this time. Water boatmen feed on algae and minute aquatic organisms. *H. regilla* larvae are known to feed on algae, detritus, diatoms, and pollen on the water surface (Province of British Columbia 2001).

This monitoring program focuses on the larval (tadpole) stage of *H. regilla.* Whether or not this is more reliable than monitoring adult frogs remains to be seen. Adult *H. regilla* disperse after breeding, but because they call throughout the year, the areas they inhabit may be relatively easy to find. However, they usually cease calling when threatened and they have the ability to throw their voices to some extent making it difficult to pinpoint their location (Province of British Columbia 2001). Future studies might examine how the number of tadpoles in our study pond correlates with the number of adults found in the meadow.

**Acknowledgements**

We would like to thank our instructor; Dr. Edward F. Connor of San Francisco State University, for inspiring us to ask questions and to monitor for something other than monitoring's sake.

**Literature Cited**

Province of British Columbia. 2001. B.C. Frogwatch Program, Pacific Tree frog. (September 2003) [http://wlapwww.gov.bc.ca/wld/frogwatch/whoswho/factshts/pactree.htm].

De Vito, J., D.P. Chivers, J.M. Kiesecker, L.K. Belden, and A.R. Blaustein.1999. Effects of snake predation on aggregation and metamorphosis of Pacific Tree frog, *Hyla regilla.* J. Herpetol. 3: 504-507.

Dupont, W.D. and W.D. Plummer. 1997. PS power and sample size program available for ree on the internet. Controlled Clinical Trials 18: 274.

Gaudin, A.J. 1965. Larval development of the Tree Frogs *Hyla regilla* and *Hyla californiae*. Herpetologica 21:117-130.

Goin, C.J., O.B. Goin and G.R. Zug. 1978. Introduction to Herpetology. W.H. Freeman and Company, San Francisco.

Huybrechts, C. 2001. The biogeography of Pacific Tree Frog (*Hyla regilla*). San Francisco State University, Department of Geography.

Marco, A., C. Quilchano and A.R. Blaustein. 1999. Sensitivity to nitrate and nitrite in pond breeding amphibians from the Pacific Northwest, USA. Environ. Toxicol. Chem.12: 2836-2839.

Nussbaum, R.A., E.D. Brodie, Jr. and R.M. Storm. 1983. Amphibians and reptiles of the Pacific Northwest. The University Press of Idaho, Idaho.

Hollingsworth, B. and K. Roberts. 2000. Field Guide: Amphibians. San Diego Natural History Museum. (Sept. 2003) [http://www.sdnhm.org/fieldguide/herps/hyla-reg.html].

Snedecor, G.W. and W.G. Cochran 1980. Statistical Methods. 7th Edition. Iowa State University Press, Ames, IA.

Stebbins, R.C. 1985. A field guide to western reptiles and amphibians. Boston, MA.

**Appendix A: raw data**

TADPOLE COUNTS

| D/S | Depth (cm) | Tadpole count | Aborted count |
|---|---|---|---|
| **D** | **22.0** | **44** | **32** |
| **D** | **25.0** | **9** | **5** |
| **D** | **37.0** | **9** | **4** |
| **D** | **20.0** | **51** | **27** |
| **D** | **24.0** | **37** | **26** |
| **S** | **5.5** | **24** | |
| **S** | **4.5** | **47** | |
| **S** | **6.0** | **76** | |
| **S** | **2.5** | **2** | |
| **S** | **5.0** | **6** | |

POND MEASUREMENTS

| measurement # | Pond width (m) | Deep width (m) |
|---|---|---|
| 1 | 8.5 | 3.0 |
| 2 | 9.5 | 10.5 |
| 3 | 19.5 | 11.1 |
| 4 | 25.0 | 11.3 |
| 5 | 26.0 | 9.0 |
| 6 | 30.4 | 6.9 |
| 7 | 29.3 | |
| 8 | 30.8 | |
| 9 | 30.0 | |
| 10 | 29.8 | |
| 11 | 27.0 | |

**Appendix B: Generalized Formulas for Analysis of Stratified Sampling**

If $X$ is the variable to be analyzed,
and $M$ is the number of strata
and $N$ is the number of sampling units that exist in the population
and $N_j$ is the number of sampling units that exist in stratum $j$
and $n$ is the number of samples taken overall
and $n_j$ is the number of samples taken from stratum $j$
and $s_j$ is the estimated standard deviation of stratum $j$
then

Estimate of the mean for the population:

$$\bar{x} = \sum_{j=1}^{M} \frac{N_j}{N} \bar{x}_j$$

Estimate of the standard deviation for the population:

$$s = \sqrt{\sum_{j=1}^{M} \left[ \left( \frac{N_j}{N} \right)^2 s_j^2 \left( \frac{N_j - n_j}{N_j} \right) \right]}$$

Estimate of the standard error for the population:

$$s.e. = \sqrt{\sum_{j=1}^{M} \left[ \left( \frac{N_j}{N} \right)^2 s_j^2 \left( \frac{N_j - n_j}{N_j} \right) \frac{1}{n_j} \right]}$$

Optimal Allocation of samples:

The samples should be allocated to the strata in proportion to the following value for each stratum:

$\frac{N_j s_j}{\sqrt{\text{cost}_j}}$, where $\text{cost}_j$ is the cost of sampling from stratum $j$.