A Skeletochronological Study of the Age Structure, Growth, and Longevity of the Mountain Yellow-legged Frog, *Rana muscosa*, in the Sierra Nevada, California

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We used skeletochronology to determine the ages of 149 (74 females, 44 males, and 31 juveniles) Mountain Yellow-legged Frogs (*Rana muscosa*) from 13 locations (elevation 1509–3501 m) throughout their current range in the Sierra Nevada mountains of California. Lines of arrested growth (LAGs) from excised toe bones were distinct in these high elevation frogs, and each LAG was assumed to represent one year of age. Females ranged in age from 0–10 years (mean = 4.1 years) and males from 0–8 years (mean = 4.0 years). The skeletochronological age was that of the post-metamorphic frog and did not include the tadpole stage. Mountain Yellow-legged Frogs spend 3–4 years as tadpoles, but no age markers are found in their cartilaginous skeletons; thus, their total age, if both tadpole and post-metamorphic stages were included, would range up to 14 years. Females were significantly longer (snout–vent length: SVL) than males and had greater mean mass, but there was no difference in the mean ages. Juvenile frogs of unknown sex ranged in age from 0–3. The von Bertalanffy growth curve demonstrated that female SVLs were larger than males for all ages. Using a semi-parametric growth model, we also found that elevation within the Sierra Nevada range was an important variable in the relationship between SVL and age; frogs from lower elevation sites were consistently larger at a given age when compared to higher elevation sites. For each increase of 1000 m in elevation, the estimated length (on average) decreases by 8.7 mm. This is the first age determination study of a Sierra Nevada amphibian, and compared to other anuran species, Mountain Yellow-legged Frogs were found to be relatively long-lived, which will have implications for restoration and recovery plans.

The Mountain Yellow-legged Frog, *Rana muscosa*, is endemic to the Sierra Nevada mountain range of California and was historically a common inhabitant of lakes and ponds at 1400–3700 m (Grinnell and Storer, 1924). Despite the fact that its habitat is protected within national park and national forest wilderness areas, *R. muscosa* is now extirpated from more than 50% of its historic localities (Drost and Fellers, 1996; Jennings, 1996). It was recently found warranted but precluded for federal Endangered Species Act protection (U.S. Fish and Wildlife Service, 2003). Restoration and recovery plans and viability models will require a better understanding of the demography of *R. muscosa*, including information on the age structure and longevity of this frog. Little is known regarding the lifespan or age structure of any Sierra Nevada amphibian.

Skeletochronology is an effective tool and has been widely used for over 20 years to evaluate age and growth in amphibians (Castanet and Smirina, 1990; Smirina, 1994; Esteban et al., 1996). Annual seasonal changes and the physiological response of amphibians cause the formation of bone growth marks, including zones of thicker layers of bone laid down during periods of fast osteogenesis, and lines of arrested growth (LAGs) formed during periods when osteogenesis is slow or inactive (Castanet and Smirina, 1990). A zone followed by a LAG typically corresponds to a one-year cycle of activity in cold or temperate regions and has been confirmed as an annual mark for several amphibians (Smirina, 1994).

Due to the extreme environment encountered at high elevations (e.g., long winters, sustained freezing, and low temperatures), *R. muscosa* are only active for a few months during the summer after snowmelt and before the winter freeze (Pope and Matthews, 2001). High elevation amphibian growth is punctuated because the active season is short, thus skeletochronology should be effective for high elevation species like *R. muscosa*. The method has been effectively used to determine ages of many ranids (Leclair and Castanet, 1987; Leclair et al., 2000; Tsiora and Kyriakopoulou-Sklavounou, 2002), including...
high elevation species (Miaud et al., 1999; Reaser, 2000), but has not been applied to any Sierra Nevada amphibians. This technique requires collection of a long bone, such as a toe, to be analyzed for LAGs.

The objective of this study was to use skeletochronology (toe bones) for age determination in *R. muscosa*. By sampling frogs of all sizes throughout the current range in the Sierra Nevada, we also investigated how specific variables such as sex and elevation are related to their growth or longevity.

**Materials and Methods**

To estimate ages of *R. muscosa*, toes were collected from frogs at 13 study sites throughout the Sierra Nevada (Fig. 1). Each frog was measured for snout–vent length (SVL) in mm using calipers, mass (g) was recorded using Pesola spring scales, and each frog was sexed (adult male, adult female, or juvenile). Sex was determined by the enlarged nuptial pad at the base of the inner-most finger found on males. If a frog was greater than 40 mm (SVL) and had no nuptial pad, it was categorized as a female. Frogs less than 40 mm SVL could not be sexed and were categorized as juveniles. A portion of the 4th digit on the right hindlimb was removed, and the frog was then released. We excised bones by cutting at a point that left the two most distal phalanges and their two associated joints completely intact. The sample was placed in a labeled envelope for later lab processing; no special storing of the bone was required. The section of the phalanx that is suitable for aging by counting annual layers was very short. Closer to the epiphyses, the thinner-walled and younger bone is mostly cartilage and entirely lacking in annuli. The specific bone section used for age determination was the second, most distal phalanx, and it was important to include the joints between the 1st (distal) and the 3rd (proximal) phalax in each sampled toe bone. The sectioning point was then selected to begin just proximal to the midpoint. A series of six sections were taken at intervals that extended distally.

The toes were prepared for sectioning by isolating the second phalanx and measuring the width of this bone to the nearest 0.25 mm. The bone was then decalcified and embedded in a paraffin block (Leclair and Castanet, 1987). Six 14-micron sections were made on a rotary microtome at intervals calculated to include a section at the exact mid-diaphysis of the bone. The sections were mounted onto microscope slides and stained with hematoxylin. The slides were coverslipped using a toluene-based resin, creating a permanent mount. Magnifications of 100–250× were used to examine the toe sections.

Assumptions in the standardized analysis for *R. muscosa* were that LAGs form during winter when activity is reduced, and the first LAG is formed during the first winter of life after metamorphosis (Leclair and Castanet, 1987). There is no LAG deposition during the aquatic tadpole phase because the skeleton is cartilaginous and younger tadpoles lack limbs. The absence of LAGs in three-year-old American Bullfrog tadpoles has been reported (Khonsue and Matsui, 2001). Thus, the skeletochronological age is that of the adult frog and does not include the tadpole stage. *Rana muscosa* tadpoles overwinter for at least two to three years (Zwiefel, 1955; Cory, 1963; Bradford et al., 1993), up to four years at higher elevations (Matthews, pers. obs.), and metamorphosis typically occurs in late summer. At lower elevations metamorphosis may occur after 1–2 years as a tadpole (Storer, 1925).

In amphibians, inner bone layers and LAGs may be lost (endosteal resorption) in older animals (Smirina, 1994). Uncertainty about the number of years of growth resorbed is a source of age analysis error. Because the season of most rapid growth of the adult frog is shortest during the year of metamorphosis, the bone formed in the summer/fall of the year of metamorphosis (located internal to the first LAG) is thinner than bone of the following year. Because the bone layer of the first year is thinnest, it is the most likely to be resorbed in older frogs. In the oldest frogs, more than one bone layer may be absent.

We attempted to identify the first layer present by using these criteria: 1) thickness of the layer in question; 2) thickness of the layer external to it; and 3) presence of proximal (3rd section of phalanx) peripheral bone. The exact time of year at which bone growth resumes is not well known. The best method for locating the first LAG is to observe the bones of recently metamorphosed frogs. In these bones, the first LAG without resorption is identifiable. By measuring the distance of the first LAG from the center of the medullar cavity, comparison with bones of larger frogs can determine whether the first and subsequent LAGs are eroded. For the purpose of age analysis, we assumed that the peripheral layer of bone that was formed by July would represent only a part of the annual increment and would be very thin, particularly in older frogs. For example, a frog determined to have eight LAGs (Fig. 2) includes seven identifiable LAGs and one resorption area.
It was assumed that the number of LAGs reflected chronological age of the metamorphosed frogs (juveniles and adults). All toe samples were processed, and LAGs were counted by a commercial laboratory (Matson’s Laboratory, Milltown, MT) after an initial consultation with Gary Matson to identify LAGs and ensure agreement on age estimates. After we received the mounted slide samples, we also confirmed age estimates. Ages were estimated by counting the first identifiable line of arrested growth (LAG) that was preceded by a well-developed layer of bone. The LAG of the first winter of life is assumed to be within about ten microns of the medulla of the bone and is likely to be partially or totally resorbed in animals older than one year. Total age was estimated by adding 3–4 years of tadpole life to LAG counts.

We tried to sample frog toes from representative areas reflecting the current distribution from the northern Sierra Nevada (Plumas National Forest) to Kings Canyon National Park in the south (Fig. 1), and also from a range of elevations. To minimize damage to small populations, we restricted our sampling to areas that had more than 15 adults. We attempted to sample

![Figure 1: Map of sites throughout the Sierra Nevada where *Rana muscosa* toes were sampled.](image-url)
though November–December (Matthews, unpubl. data). Some frogs at the lower elevation were also found in streams while higher elevation frogs were typically in lakes and ponds.

To explore the relationship between age and length (SVL), we started by fitting the widely used von Bertalanffy (von Bertalanffy, 1938) function describing SVL as a function of age. The von Bertalanffy growth function is used extensively in fisheries research and has now been used in several amphibian growth studies (Miaud et al., 2000; Cogalniceanu and Miaud, 2003):

\[
L(t) = \text{L}_{\text{max}} - (\text{L}_{\text{max}} - \text{L}_{\text{min}}) \exp\left[-K(t-t_0)\right],
\]

where \( t = \text{age} \) (an integer of the number of LAGs), \( L(t) = \text{SVL} \) at time sampled (age \( t \)), \( t_0 = \text{offset to 0.5 years old} \) to account for not sampling true age 0, \( \text{L}_{\text{max}} = \text{maximum SVL} \), SVL of frog for infinite age, \( \text{L}_{\text{min}} = \text{SVL} \) at one year old, \( K = \text{growth rate} \) (specifically the von Bertalanffy growth coefficient) or the rate at which growth reaches \( \text{L}_{\text{max}} \). Differences in growth coefficients and \( \text{L}_{\text{max}} \) were compared using t-tests. We fit equation/model [1] to data from all sites, but separately for males and females; juvenile frogs were not included.

Because the growth curves may be different among the 13 sites due to elevation, we also fitted a semi-parametric regression model (model 2, Hastie et al., 2001) to determine whether elevation was affecting size at a given age. For example, frogs at lower elevations might be larger due to a longer summer activity period (typically from May–November) during the year cycle. In the Sierra Nevada the lowest elevations where \( \text{R. muscosa} \) are currently found include the northern and western sites (Fig. 1). In contrast to the von Bertalanffy growth model, this model did not assume any specific shape for the growth curve. The shape of the growth curve was estimated from the data using cubic spline functions:

\[
\text{Model } [2] \quad \text{L} = \text{sex} + s(\text{age, sex}) + \text{elevation} + \text{error},
\]

where \( \text{L} = \text{SVL of R. muscosa} \), sex = a categorical variables with 3 levels (female, male, juvenile), age = estimated age using LAGs, elevation = elevation of the site where the frog was captured, error = independent random error terms, \( s(\cdot) \) = spline function describing the nonlinear relationships between the explanatory variables and the response L. This model was then used to determine if there were differences in male and female SVL at a given age and if there were differences in SVL at age throughout the sampling area. These analyses were done only using male and female data (no juveniles).

Fig. 2. Photograph of toe cross section showing eight lines of arrested growth (LAGs) for \textit{Rana muscosa}. Scale bar = 25 μm.
RESULTS

From the 162 frogs sampled, a total of 149 (74 females, 44 males, and 31 juveniles) were aged; the remaining were damaged or had inadequate bone sections that did not allow for proper analysis. Females ranged in age from zero (no LAG) to ten years and had a mean age of 4.1 (Table 1). Males ranged in age from 0–8 years and had a mean age of 4.0. The number of males, females, and juveniles in each age group are found in Table 2. When estimated tadpole years (3–4 yr) are added, longevity extended to 13–14 years in females and 11–12 years in males. The females were larger than males at all ages (Fig. 3). Females were significantly longer than males (mean SVL 563.6 vs. 56.1, t-test: P < 0.001, df = 116) and had greater mean mass than males (32.5 g vs. 21.3 g, t-test: P < 0.001, df = 116), but there was no difference in the mean ages (t-test: P = 0.882). Juvenile frogs of unknown sex ranged in SVL from 20–40 mm, in mass from 1.5–7 g, and in age from 0–3 (Table 1).

Table 1. Summary of SVL, Mass, and LAGs (Ages) of Mountain Yellow-legged Frogs Sampled in the Sierra Nevada.

<table>
<thead>
<tr>
<th></th>
<th>SVL (mm)</th>
<th>Mass (g)</th>
<th>#LAGs (age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>40</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>max</td>
<td>87</td>
<td>76</td>
<td>10</td>
</tr>
<tr>
<td>mean ± S.E.</td>
<td>63.6 ± 1.3</td>
<td>32.5 ± 1.9</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>42</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>max</td>
<td>68</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>mean ± S.E.</td>
<td>56.1 ± 1.0</td>
<td>21.3 ± 1.2</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Juveniles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min</td>
<td>20</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>max</td>
<td>40</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>mean</td>
<td>31.6 ± 0.8</td>
<td>3.8 ± 0.3</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

The von Bertalanffy growth curve (Fig. 3) demonstrates the differences between SVLs for males and females at all ages, but showed great variability at each age. Female SVLs were larger than males for all ages. Females had larger Lmax than males (69.0 mm vs. 59.9 mm, t-test: P < 0.001). The growth coefficients (K) were not significantly different between males and females (K = 0.50 ± 0.30 for females, 0.41 ± 0.30 for males; t-test: P = 0.10).

Because of the considerable variation in size at a given age (Fig. 3), we explored the possibility that elevation may influence growth, and the semi-parametric regression model 2 indicated that elevation was important in accounting for some of the variation between observed and fitted SVLs (adjusted R² = 0.78, non-parametric regression; Fig. 4; model 2 P-values: Psex < 0.0001, Psex(age,sex) < 0.0001, Pelevation < 0.0001).

Table 2. Summary of the Number of Males, Females, and Juveniles in Each Age Category.

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
<th>Juvenile</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>12</td>
<td>16</td>
<td>31</td>
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<td>2</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>9</td>
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<td>19</td>
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<td>7</td>
<td>4</td>
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<td>11</td>
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<td>4</td>
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<td>9</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>74</td>
<td>31</td>
<td>149</td>
</tr>
</tbody>
</table>

Fig. 3. Fitted growth curves using model 1 (von Bertalanffy growth model) with length (SVL) and age data for male and female frogs. Ages do not include tadpole phase. We offset t₀ = 0.5 years to account for not sampling true age 0.
We found a mean average deviation of 5 mm between the observed and fitted values. The slope of the elevation  0.0087 (S.E. 0.0012); for each increase of 1000 m in elevation, the estimated length (on average) decreases by 8.7 mm. The scatter plot (Fig. 5) shows the relationship between elevation and SVL; SVL decreases as elevation increases. This was a pattern across sampling sites for both males and females to be larger at a given age at lower elevation sites. On average males were between 7.2–12.7 (95% CL) mm smaller than females.

**DISCUSSION**

This is the first study using skeletochronology to estimate the ages of a Sierra Nevada amphibian. Because *R. muscosa* spend an extended number of years as tadpoles (3–4 years), total age, including both tadpole and adult stages, ranged up to 14 years. Compared to other high elevation amphibians, *R. muscosa* is long-lived. Although no age determination techniques were used, Sherman and Morton (1993) estimated from tag recoveries that some female *Bufo canorus* were at least 15 years old from field sites near Tioga Pass in the central Sierra Nevada (~3000 m). Reaser (2000) used skeletochronology to determine age of post-metamorphic *R. luteiventris* from the Toiyabe range in Nevada (elevations 2200–2700 m) and estimated that ages ranged up to seven years for females and up to five years for males. In lower elevation areas, Cogalniceanu and Miaud (2003) reported ages of 4–10 years for individuals in the *R. esculenta* complex from the Danube River floodplains. Skeletochronology was also successful for determining ages of Wood Frogs (*R. sylvatica*); ages up to five years were reported from Quebec (Bastien and Leclair, 1992).

Like most anurans, *R. muscosa* are sexually dimorphic (Monnet and Cherry, 2002); *R. muscosa* females were larger than males, similar to that seen in other ranid species (Reaser, 2000; Monnet and Cherry, 2002). From our studies in Kings Canyon National Park we knew that female *R. muscosa* were typically larger (SVL: mean = 63.5 mm for females, mean = 55.9 mm for males, n = 1250 frogs, Matthews, unpubl. data) and heavier than males. The smaller sizes of males and possible higher vulnerability to predation may contribute to their shorter lifespan, although more data are needed to confirm. Only one frog was determined to be ten years (3501 m), and no nine-year-old frogs were found, suggesting that survival to the older ages is rare. However, in 2006 we caught 44 frogs (24 females and 20 males) of the 356 adult frogs originally tagged with PIT tags in Dusy Basin, Kings Canyon National Park in 1997 (Matthews, unpubl. data); these frogs were at least ten years old (total age 12–13 years). Although males of other species often are more conspicuous and prone to predation when groups of males aggregate and form large chorusing groups during breeding (Shirose et al., 1993; Tsiora and Kyriakopoulou-Sklavounou, 2002), male *R. muscosa* lack vocal sacs (Stebbins, 2003), do not call during breeding, and do not form breeding aggregations. Garter snakes and introduced trout are common amphibian predators throughout the Sierra Nevada (Jennings et al., 1992; Matthews et al., 2002) and may target males because of their smaller size.

At lower elevations growth was faster and frogs were bigger at most ages. This difference is likely due to the longer summer; at sites in the northern Sierra Nevada (elevation 1509–1872 m) frogs are active from April/May through November. In contrast, lakes and water bodies at higher elevations (>2500 m) are frozen or covered in snow for longer periods and keep frogs snowbound for extended periods; thus, they have a shorter active period, typically from late June through early October (Matthews and Pope, 1999; Pope and Matthews, 2001). These shorter active periods reduce feeding times, resulting in reduced growth rates.

Skeletochronology was effective in age determination of *R. muscosa*. Mountain Yellow-legged Frogs are relatively long-lived anurans,
and those at lower elevations were larger at a given age. This study demonstrated the need to sample across the frog’s current distribution as there were considerable differences in age and growth throughout the Sierra Nevada. Because of their age distribution across elevations, restoration and management of *R. muscosa* will require preserving habitat across a wide range of elevations.

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**LITERATURE CITED**


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