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# Seasonal Activity, Population Characteristics, and Age Estimation in the Aquatic Salamander, Siren intermedia nettingi (Goin)

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# Seasonal Activity, Population Characteristics, and Age Estimation in the Aquatic Salamander, *Siren intermedia nettingi* (Goin)

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## Abstract

We conducted a study of the Western Lesser Siren (*Siren intermedia nettinigi*), at a locality termed the Airport Road site in Jonesboro (Craighead County, AR) from November 2004 until March 2007. This site consisted of a network of roadside ditches in cultivated lawns in an industrial park. Even though sirens are known to occur frequently in ditches, most *studies* of the genus *Siren* have taken place in natural wetlands. We compiled mark-recapture data at the Airport Road site for each season to determine if the seasonal activity pattern for sirens in northeast Arkansas varied from activity data previously published from other localities in the range of this species. Capture rates were higher in the fall and spring. The predicted overall population size was 110 sirens at a density of 0.81 sirens per linear m. This density was less than the densities (in sirens/$m^2$) reported by previous studies. We found two prominent peaks in sirens per size class: the first at 161-170 mm, and the second at 201-210 mm. Other researchers have assumed that the two most abundant size classes in siren populations represent one-year-old and two-year-old cohorts. The sirens captured at the Airport Road site are smaller, on average, than those reported in previous population studies. We found no significant difference between the growth rates of sirens larger than 200 mm SVL and those smaller than 200 mm snout-vent length (= SVL; *P* = 0.957, confidence interval -1.945, 2.045, n = 16). Our mean growth rates did not significantly differ from growth rates reported for sirens elsewhere. We sectioned siren humeri to identify and quantify lines of arrested growth (LAGs) as part of a skeletochronological analysis. The use of SVL was a poor indicator of number of LAGs. The difference in the weather pattern history in each of the voucher sirens used likely resulted in broad ranges of LAGs for each SVL size class.

## Introduction

The Western Lesser Siren, *Siren intermedia nettingi,* is rarely encountered even though this animal can be found in high numbers in suitable habitats, such as agricultural and roadside ditches or wetlands managed for waterfowl (Frese et al. 2003). Sirens have been found to occur in higher densities than any other salamander including all terrestrial salamanders except for the San Marcos Salamander, *Eurycea nana* (Gehlbach and Kennedy 1978, Tupa and Davis 1976). The Western Lesser Siren is a relatively large amphibian; adults range from 18 to 50 cm in total length (Conant and Collins 1998). Males are typically larger than females (Sugg et al. 1988). External gills are present anterior to the sole pair of forelimbs and coloration is variable ranging from olive-green to blue-gray (Trauth et al. 2004). Sirens are highly fecund, typically producing around 100-300 eggs and in somecases over one thousand ova can be produced (Gehlbach and Kennedy 1978, Trauth et al. 2004). Gehlbach and Kennedy (1978) suggest that sexual maturity is likely attained in one year for both sexes of sirens; however, Trauth et al. (1990) reported size class data illustrating that females reproduce at two years of age.

In a natural drainage, sirens appear to feed opportunistically by filter feeding in the mud and by randomly ingesting pieces of aquatic plants to obtain small animals occurring upon the plants (Altig 1967). Sullivan et al. (2000) reported that *Siren intermedia* are random suction feeders which do not rely heavily upon visual or chemical cues during foraging. The relative dearth of information on sirenids prompted us to study sirens in an urban setting. Comparisons of population characteristics between our study and past studies are useful in assessing the effect of urbanization on sirens and aquatic salamanders in general. Age data, determined through skeletochronological analysis, further illuminates population characteristics found at Airport Road.

*Seasonal activity*.—Whereas many salamanders aestivate during periods of sustained heat and/or drought, sirenids have a unique repertoire of behaviors and structures that allow them to withstand longer periods of aestivation when the aquatic environment dries (Petranka 1998). During periods of drought, sirens can burrow into the substrate and survive for extended periods of time until rains inundate the habitat (Gehlbach et al. 1973). Once inundated with water, an aestivating siren will become active in about one day; however, its activity levels will peak only after feeding (Gehlbach et al. 1973). Since the Western Lesser Siren inhabits areas where there is a dramatic seasonal fluctuation in precipitation and temperature, these animals may have multiple periods of inactivity per year (Petranka 1998). As the temporary water in pools, ponds, or ditches dries up in the summer, sirens must aestivate (Trauth et al. 2004). When temperatures become too low for poikilothermic activity in the winter, sirens become torpid (per. obs.). It is not known at what temperature sirens become inactive. However, it is intuitive that sirens will become inactive when the liquid water completely dries or freezes in their habitat.

*Population size and density.*—Sirens in natural drainages have been reported to occur in high densities (Altig 1967). Sirens in a Missouri wetland occurred at a standing crop biomass of 1.35 to 2.17 sirens/m$^2$ with 44.9 to 72.2 grams/m$^2$ (Frese et al. 2003). In that study, home ranges of individuals varied in size and overlapped frequently. Ditches are structurally different from the wetland mentioned above. Ditches have greater length with a relatively narrow width, whereas the Missouri wetland is mostly equal in size between its two dimensions (B. Wheeler, pers. comm.); however, we expected the population density of *Siren intermedia nettingi* at the Airport Road site to be similar to the standing crop biomass of 1.35 to 2.17 sirens/m$^2$ reported by Frese et al. (2003).

*Size classes*.—Gehlbach and Kennedy (1978) found that four distinct size classes occur at 1-20 g, 21-50 g, 51-70 g, and 71-160 g. In five-year-old beaver ponds in Texas, the largest male measured snout-vent length (SVL) 313 mm, total length (TL) 465 mm, and mass 265.4 g. The largest female measured SVL 215 mm, TL 321 mm, and mass 76 g (Gehlbach and Kennedy 1978). The record size *Siren intermedia nettingi* measured 502 mm in total length (Conant and Collins 1998). However, in unpublished records, Western Lesser Sirens measuring up 630 mm TL have been collected (McDaniel 1969). We aimed to determine if the low diversity of vascular plants and spatial nature of the urban ditch network at the Airport Road Site had an effect on sizes and size class distribution of these animals.

*Growth rates.*—The Western Lesser Siren is a relatively large amphibian; adults range from 18 to 50 cm in total length (Conant and Collins 1998). Male Western Lesser Sirens are typically larger than females (Sugg et al. 1988). Gehlbach and Kennedy (1978) suggested that sexual maturity is likely attained in one year for both sexes of sirens; however, Trauth et al. (1990) report size class data illustrating that females first reproduce at two years of age. Sexes occur in a 1:1 ratio (Gehlbach and Kennedy 1978). Regardless of sex, first year sirens grow more rapidly than mature individuals (Frese et al 2003). An accurate method for determining the sex of live sirens has not yet been documented, but sex can be determined confidently upon dissection of euthanized animals. We aimed to determine if sirens from an urban setting grew at similar rates to those from more natural areas. Such a determination is important to estimate the impact of urbanization on aquatic salamanders in general.

*Skeletochronological analysis.*—As periosteal growth occurs, new layers of bone are deposited on endochondral long bones (Zug 1991). Within cross sections of these bones, lines of arrested growth (LAGs) can be counted to infer age. Lines of arrested growth are caused by a cessation in growth due to inactivity and a halt in feeding. The zones in between the LAGs represent periods of activity and regular feeding (Zug 1991, Wake and Castanet 1995).

## Materials and Methods

### *Study area*

We studied a population of *Siren intermedia nettingi* at a specific ditch site in Jonesboro, Arkansas along Airport Road (35˚ 49' 50.4 " N, 90˚ 39' 36.7" W). The interconnected ditch network is adjacent to industrial facilities such as warehouses, metal working factories, and masonries. There are five ditches all within 150 m of each other and all potentially contiguous when water levels are high. These ditches were designated site 1-5 from north to south. Although formerly a floodplain, the surrounding area now consists of large treeless lawns of regularly maintained turf grasses.

***Field methods***

The Airport Road site was monitored from November 2004 until March of 2007. Sirens were collected from the site using dip nets and traps. When water levels in these ditches were high, >25 cm, we trapped daily; traps were checked in the morning and were baited if necessary in the evening. The traps were left out permanently until the water level became too low. These cylindrical traps are typically made of rubber-coated steel. Commercially available traps such as these are quite reliable at catching and retaining snakes, *Amphiuma,* and sirens (Wilson et al. 2005).

***Seasonal activity***

The date of collection was recorded for each siren captured. These data were represented graphically to illustrate trends in seasonal activity. We followed Raymond (1991) and conducted a pairwise comparison of mean number of siren per month collected per season. Using Minitab 14®, we employed an Analysis of Variance and a Tukey's Pairwise comparison test to compare seasonal activity. Since the duration of our study does not represent three complete years, we chose to represent mean captures per month compiled from all years studied.

***Population size and density***

Captured sirens were observed for previous marks or were marked for the first time. We used visible implant elastomer dye primarily due to its ease and efficacy (pers. obs.). Care was taken to mark the animals in a manner so they could be individually recognizable upon recapture. To accomplish this, the pattern and/or color were changed with every animal marked.

***Population density estimate***

Population estimates were calculated using the Schumacher and Eschmeyer population index (Krebs 1998). This population estimate method was useful when multiple samples were collected. The equation is as follows:

$$\hat{N} = \frac{\sum_{t=1}^{s} (C_t M_t^2)}{\sum_{t=1}^{s} (R_t M_t)}$$

The variable C is the total number of sirens caught in this study and R equals the number of sirens recaptured. N represents the estimated total population at the Airport Road site while M denotes the number of marked individuals. The method for estimating standing crop biomass for sirens by trapping used in Frese et al. (2003) was employed to determine population density.

***Size classes***

Before each animal was released, we recorded the following information: date of collection, specific location, TL, SVL, mass, mark type, method of collection, weather conditions, approximate water depth, and collector(s). We assigned size classes at 10 mm increments. The size classes were graphically represented to illustrate trends such as bimodality. The size classes with the most representatives were reported. We compared the mean length of the predominant size classes and size of the largest sirens captured to the data from previous studies.

***Growth rates***

We followed the procedures used by Houck (1982) to determine growth rates based on change in snout-vent length. When sirens were recaptured and measured the amount of growth that occurred between capture events was calculated by subtracting the original SVL from the final snout-vent length. When the amount of growth was divided by the duration of time between the two capture events, an individual growth rate, irrespective of sex, was determined. The population was divided into juveniles (those not yet reproductive) and mature specimens to determine if there were a difference in growth rates between juveniles and adults as reported by Frese et al. (2003). Sirens under 200 mm SVL were designated as "small" and were compared to those designated as "large" (sirens with an SVL greater than 200 mm). A two sample T-test of unequal variance was employed to determine the difference in growth rates between recaptured *Siren* designated small and those designated as large (Minitab 14®).

***Skeletochronological analysis***

The Arkansas State University Museum of Zoology (ASUMZ) houses about 150 voucher sirens. These have all been collected from various areas in Arkansas within the last 40 years. We prepared 30 of these specimens for skeletochronological age estimates. Presnell and Schreibman (1997) provided detailed guidelines for preparing the tissue for examination. A preserved humerus with its surrounding tissue was dehydrated in an ethanol series and was cleared with xylene finally being placed in paraffin tissue blocks (Presenell and Schreibman 1997, Trauth and Worley 1997). The tissue blocks were sectioned at thicknesses between 8 and 22 µm due to the varying difficulty in tissue ribbon formation. An Erlich's hematoxylin and eosin stain was applied. Once the micrographs were generated with a Nikon Eclipse E600 light microscope, we estimated age by counting lines of arrested growth (LAGs) in the humerus cross sections at 200X magnification (Parham et al. 1996) (Figs. 4-9). We used a correlation test (Minitab 14®) with regression analysis (alpha level = 0.05) to test the relationship between SVL (mm) and number of LAGs (Sokal and Rohlf 1981).

## Results

### *Seasonal activity*

In early spring, more sirens were collected than in the preceding months of winter. We found no significant difference between the number of sirens captured in the spring and fall ($P = 0.967$; $F_{1,4} = 0.00$). Although statistically insignificant, fewer sirens were caught in summer and winter as compared to spring and fall ($P = 0.069$; $F_{1,10} = 4.15$). In March, the greatest number of sirens (n = 24) were collected. During the summer, few sirens were collected. However, in June, 10 sirens were collected. In the spring, from 0 to 24 sirens were collected per month (mean 11.00 +/- 7.00). In summer, monthly collection ranged from 0 to 10 (mean 5.00 +/- 2.89). In the fall, we collected 8 to 14 sirens per month (mean 11.33 +/- 1.76). Monthly winter collection ranged from 0 to 7 (mean 2.33 +/- 2.33). We grouped all collections from each year into these calculations (Fig. 1).

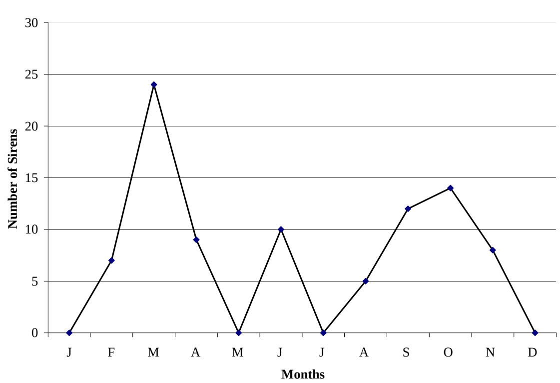


**Figure 1.** Number of sirens, *Siren intermedia nettingi*, collected from the Airport Road, Jonesboro, AR from November of 2004 to March of 2006. All values represented monthly samples inclusive of all years.

### *Population size and density*

From October 2004 to November 2006, 62 sirens were collected at the Airport Road site. Of these, 19 were recaptured at least once; seven were recaptured at least twice; three were recaptured at least three times, and one of these was recaptured a fourth and fifth time. Thirty-one percent of all sirens collected were recaptured at least once. As calculated by the Schumacher-Eschmeyer method, the estimate for total population of *Siren intermedia* in the Airport site is 110 individuals (+/- 0.00091). Since the ditches involved in this study exhibit an extreme fluctuation in depth and width, density can best be reported by animals per linear m. The trapped portion of the ditches at the Airport Road site totaled 136.6 linear m. The linear density of sirens is therefore estimated to be 0.81 sirens/m.

### *Size classes*

There is a major peak in number of sirens per size class at the 201-210 mm size class. In general, size classes between 160 mm and 240 mm had more representatives than size classes with larger or smaller sirens (Fig. 2). No sirens with a SVL of less than 13 mm were found at Airport Road. This result was expected since newly hatched sirens measure ca. 13 mm TL (Bishop 1943). The largest sirens collected had a SVL of 255 mm, smaller than the largest sirens captured by Gehlbach and Kennedy (1978) and Sugg et al.1998. The mean SVL for all animals captured in this study (181.1 mm) is less than the mean SVL for both male and female *Siren* collected by Sugg et al. (1988) 282.4 and 216.9 mm, respectively (Table 1). Our overall mean TL (277.3 mm) is less than the TL for both sexes (males 348.2 mm, females 286.8 mm) reported by Frese et al. (2003) and is less than the mean TL for male sirens (321.9 mm) reported by Sugg et al. (1988); however, the mean TL for female sirens reported by Sugg et al. (1988) is 189.5 mm, which is less than the mean TLs reported by Frese et al. (2003) and our study (Table 1). The length to mass relationship is similar to that reported in previous studies (Gehlbach and Kennedy 1978).

### *Growth rates*

Upon comparison of the growth rates of sirens under 200 mm SVL to those with an SVL of 200 mm or greater, we found that there was no significant difference between the growth rates of large and small *Siren* ($P = 0.957$; $T11 = 0.06$; n = 16). Growth rates of *Siren* less than 200 mm SVL ranged from -1.00 to 1.40 mm/day with a mean of 0.055 (SE = 0.32; n = 10). In

**Table 1.** Comparison of the mean and range of total length and snout-vent length (in mm) of Western Lesser Sirens from northeast AR (this study), central AR (Sugg 1988), southern MO (Frese et al. 2003) and southern TX (McDaniel 1969).

| N | mean SVL | SVL range | SVL SD | mean TL | TL range | TL SD | Sex | Study |
|---|---|---|---|---|---|---|---|---|
| 59 | 181.1 | 135-255 | 49.3 | 277.3 | 170-365 | 47.6 | Both | This study |
| ca. 1200 | 282.4 | 122-315 | 45.0 | 321.9 | 179-461 | 60.9 | M | Sugg et al. 1988 |
| ca. 1200 | 216.9 | 138-282 | 29.9 | 189.5 | 221-439 | 38.2 | F | Sugg et al. 1988 |
| 911 | - | - | - | 348.2 | 317-403 | 23.2 | M | Frese et al. 2003 |
| 911 | - | - | - | 286.8 | 215-365 | 32.9 | F | Frese et al. 2003 |
| 378 | - | - | - | - | 60-630 | - | M | McDaniel 1969 |
| 378 | - | - | - | - | 60-520 | - | F | McDaniel 1969 |

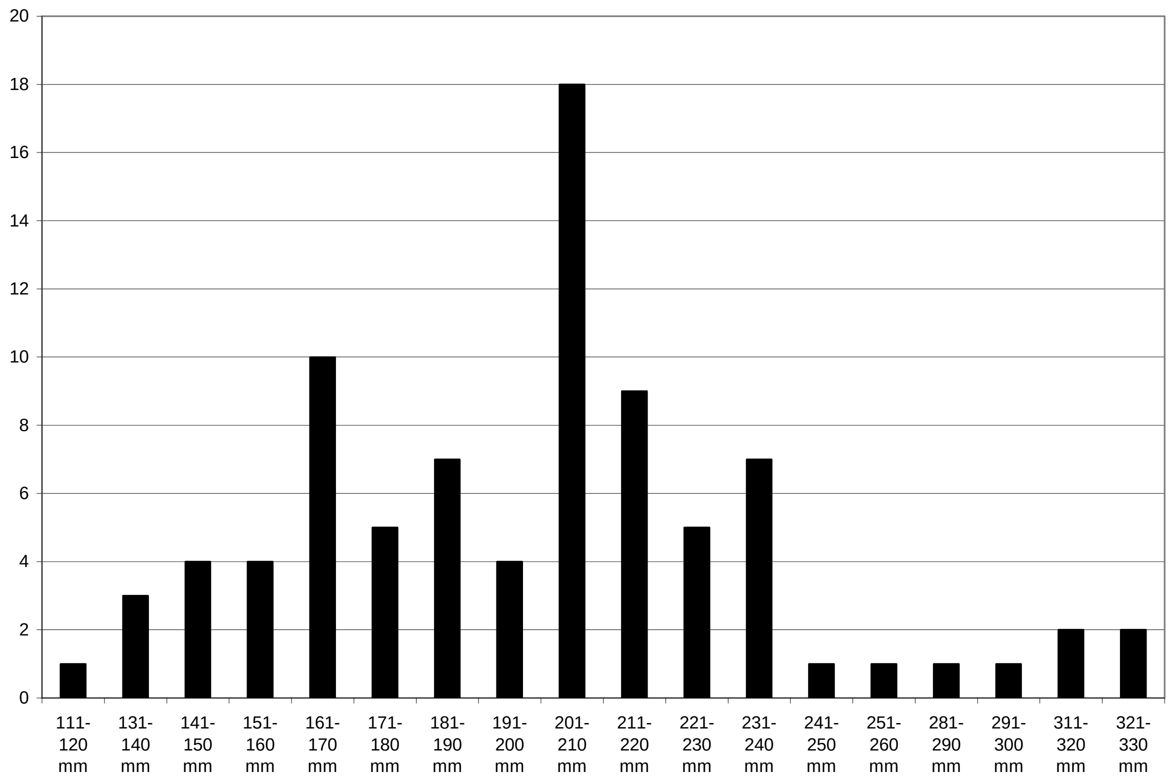


**Figure 2.** Number of representatives per size class. Data are from all sirens collected at the Airport Road site throughout the duration of this study. Size classes are based on snout-vent lengths.

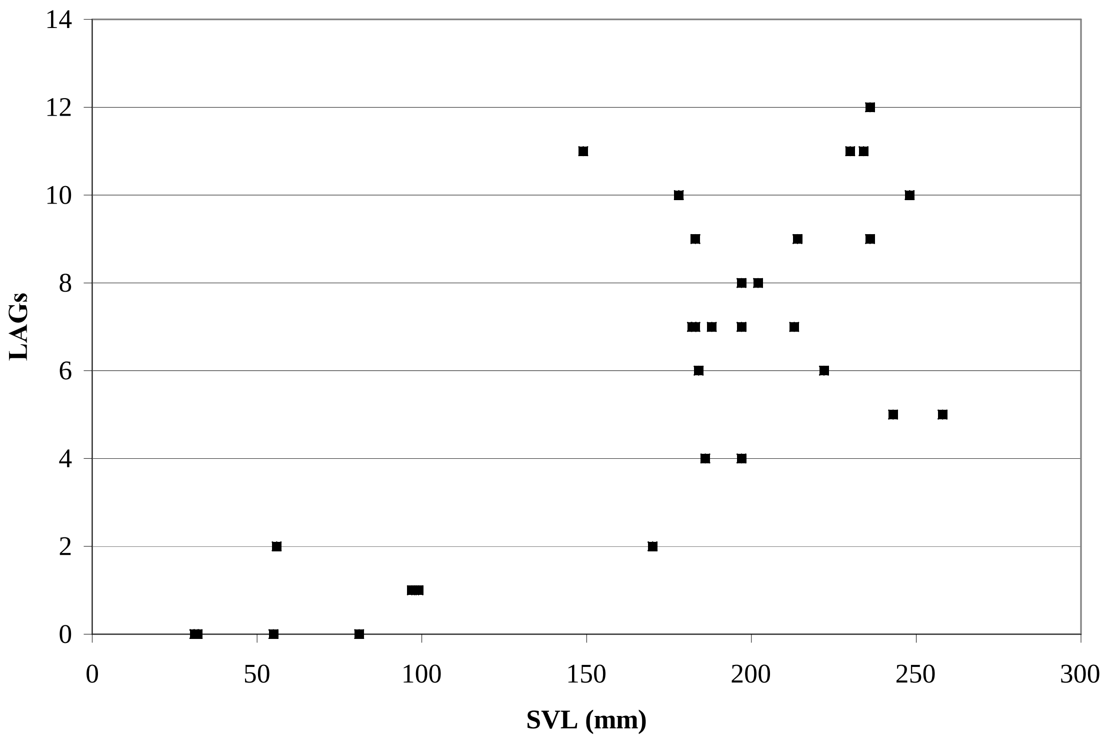


**Figure 3.** Relationship between snout-vent length (SVL) and lines of arrested growth (LAGs) in 30 *Siren* from the ASUMZ.

*Siren* larger than 200 mm, the growth rates ranged from -5.33 to 6.00 mm/day with a mean of 0.005 (SE = 0.848) (n = 6). With respect to TL (total length), growth rates of "small" *Siren* ranged from -0.24 to1.72 mm/day with a mean of 0.371mm/day (SE = 0.208) (n = 10). Growth rates of "large" *Siren* ranged from -0.15 to 2.29 mm/day with a mean 0.639 mm/day of (SE = 0.365) (n = 6). *Siren* designated as "small" exhibited a mean mass growth of -0.203 g/day (SE = 0.14) (n = 10). "Large" *Siren* grew at 0.371 g/day on average (SE = 0.23) (n = 6).

***Skeletochronological analysis***

The SVL of the 30 sirens examined ranged from 31 to 258 mm. The number of LAGs ranged from 0 to 12. By employing a correlation test with regression analysis, we found a significant correlation between SVL and LAG (P <0.001; SE coefficient =1.143). Two unusual observations could not be included in the statistical analysis. One was a siren with an SVL of 149 mm that possessed 11 distinct LAGs; the other had the other had an SVL of 258 mm and had 5 LAGs (R = 2.54 and -2.50, respectively). The relationship between LAGs and SVL is weakly positively correlated (Fig. 3); but animals measuring about 200 mm SVL, the number of LAGs is highly variable and unpredictable (see Figs. 4-9).

## Discussion

Our study of an urban population of the Western Lesser Siren at the Airport Road site represents an interesting setting for ecological study. This site hosts numerous animal species that are typically found in undisturbed natural ecosystems. Differences in population characteristics between our study and previous *Siren intermedia* studies may be attributable to the juxtaposition of the Airport Road ditch network to possible sources of pollutants such as the nearby roads and factories (pers. observ.). With respect to seasonal activity, our results seem typical; sirens are the most active in the spring and fall. The heightened spring activity may result from increased movement preceding breeding (Noble and Marshall 1932; Johnson 1977, Trauth et al. 1990). Gehlbach and Kennedy (1978) and Raymond (1991) both reported relatively high levels of activity in the spring.

The former study reported high activity in the fall, similar to our results even though that study was conducted in extreme southern Texas. This increase in activity may be a life history strategy of sirens for increasing nutrient reserves before winter torpor. However, Raymond (1991) stated that sirens exhibit no increased level of activity in fall. Perhaps those sirens were relatively more active in the winter and fall due to permanent water or mild winter temperatures in Louisiana. We found that the population density at Airport Road was less than the siren densities reported by Frese et al. (2003) and Gehlbach and Kennedy (1978). This difference may be due to factors present in urban environments not found in more natural settings. In Frese et al. (2003), sirens were studied in an intensively managed wetland and Gehlbach and Kennedy (1978) studied sirens in a natural wetland. The sirens we studied may have occurred at lower densities due to a homogeneous vegetation structure that results in a lower diversity and abundance of potential prey items. The artificial ecosystem and subsequent low prey diversity may have been responsible for the relatively small size of sirens at our site compared to previous studies (McDaniel 1969; Sugg et al. 1988, Frese et al. 2003). Each of the previous mentioned studies reports multiple sirens larger than the largest siren we collected at the Airport Road site. Low densities and smaller size classes are likely results of low prey diversity; however, we report growth rates that are not significantly different than those reported by Gehlbach and Kennedy (1978) and Frese et al. (2003). Perhaps low abundance of food at Airport Road causes low densities and smaller sizes but individual growth rates are independent (Van Buskirk and Smith 1991). We initially expected our calculated growth rates to vary from previous studies since seasonal fluctuations in weather can cause long periods of dormancy which would bring a halt to feeding and regular growth. This was not the case. In Texas, Missouri, and Arkansas, sirens grow at approximately the same rate, on average (Gehlbach and Kennedy 1978, Frese et al. 2003). However, even small fluctuations in weather apparently cause differential rates of osseous deposition in long bones. When examining siren humeri, minor LAGs were often problematic when trying to estimate age. Minor LAGs, those LAGs occurring within individual MSGs, were often nearly as dark and thick as there corresponding major LAG. Even though SVL and number of LAGs were found to have a significant correlation, it appears that skeletochronology is not a good tool for estimating age in sirens. As suggested by Eden et al. 2007, the deposition of minor LAGs due to extreme weather events and endosteal resorption limit the usefulness of skeletochronology in temperate salamanders.

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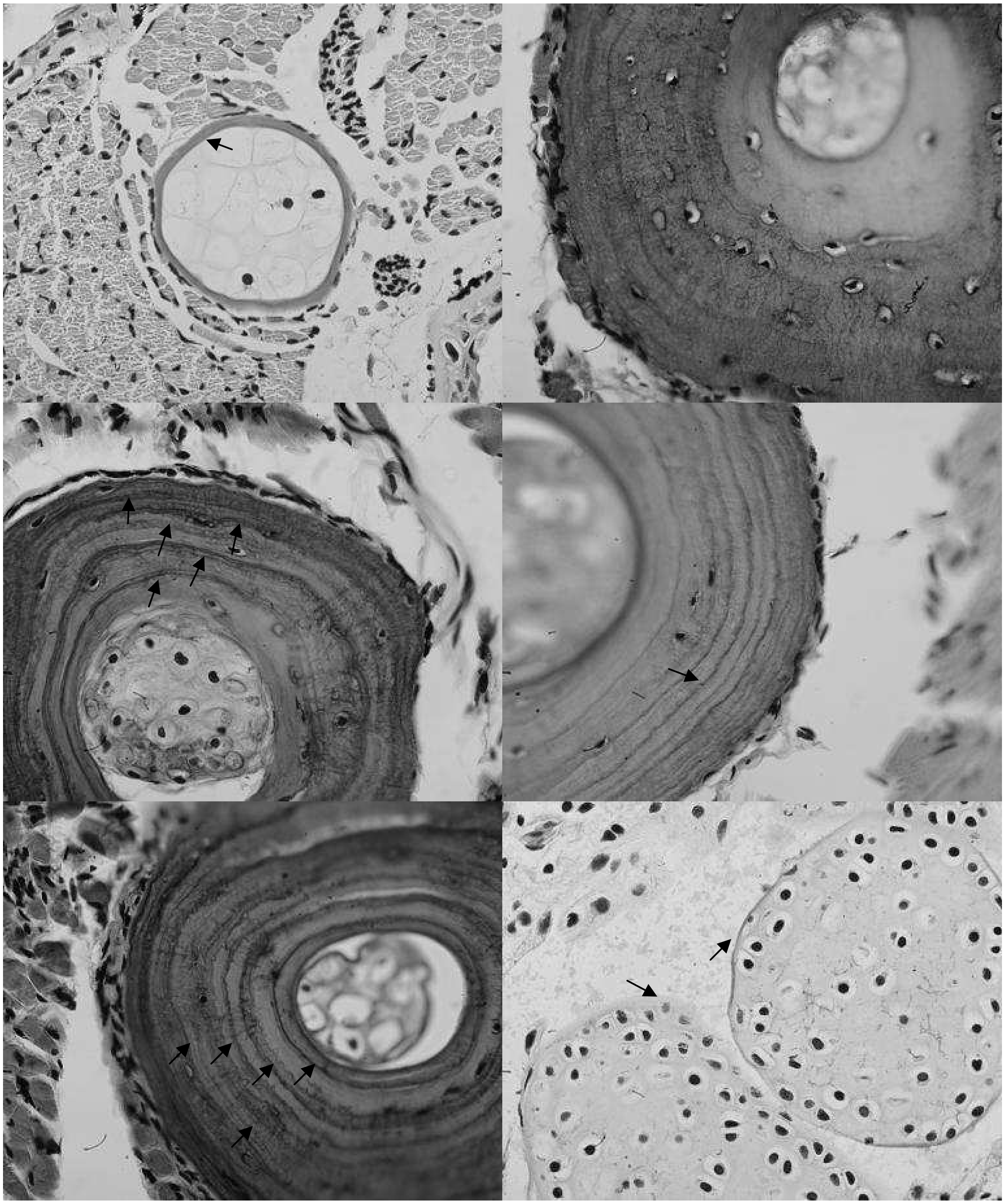

**Figures 4-9.** Clockwise from top left: 4. A humerus from a young siren showing no LAGs; 5. A humerus from an adult siren showing 5 LAGs that are not distinct; 6. A humerus with very thin MSGs and numerous LAGs; 7. The radius and ulna of a young siren; note the lack of ossified periosteum; 8. A humerus from an adult siren with 6 distinct major LAGs and numerous thin minor LAGs; 9. A humerus exhibiting apparent endosteal resorption of bone. All were photographed at 200X magnification.

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