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CONTENTS

Geographic Variation in Northern Green Frog Larvae, *Lithobates Clamitans Melanotus*, in Northwestern New Jersey
 John K. Korky and John A. Smallwood.....1

Seasonal Activity, Reproductive Cycles, and Growth of the Bronze Frog (*Lithobates clamitans clamitans*) at the Western Edge of its Geographic Range
 Walter E. Meshaka, Jr., Samuel D. Marshall and David Heinicke11

Seasonal Activity, Reproductive Cycles, and Growth of the Northern Leopard Frog, *Lithobates pipiens* (Schreber, 1782), From Pennsylvania
 Walter E. Meshaka, Jr., Pablo R. Delis, Sarah A. Mortzfeldt23

Clutch characteristics of the Southern Leopard Frog, *Lithobates sphenocephalus* (Cope, 1886), in Natchitoches, Louisiana
 Walter E. Meshaka, Jr. and Samuel D. Marshall.....36

The Effects of Temperature and Salinity on Wood Frog (*Lithobates sylvaticus*) Tadpole Growth and Survival
 Jennifer H. Clemmer, Eliza Z. Miller, Laura Wolgamott, Geoffrey R. Smith and Jessica E. Rettig38

Body temperatures of *Hyla arenicolor* from Sierra de Tepozotlan, Estado de Mexico, Mexico
 Felipe Correa-Sánchez, Geoffrey R. Smith, Guillermo A. Woolrich-Piña, and Julio A. Lemos-Espinal.....42

Clutch characteristics of the Pickerel Frog, *Lithobates palustris* (LeConte, 1825), in Natchitoches, Louisiana
 Walter E. Meshaka, Jr. and Samuel D. Marshall.....45

Distribution of Tadpoles (*Hyla arenicolor*) in the Ponds Associated To Río Salado, Puebla, Mexico
 Guillermo A. Woolrich-Piña, Julio A. Lemos-Espinal, Geoffrey R. Smith, Raymundo Montoya-Ayala and Luis Oliver-López.....47

Mississippi Map Turtle, *Graptemys pseudogeographica kohnii*, Documented in Frederick County Maryland
 Wayne G Hildebrand.....51

Reproduction in Clark's Spiny Lizard, *Sceloporus clarkii* (Squamata: Phrynosomatidae) From Sinaloa, Mexico
 Stephen R. Goldberg53

Harassment/Predation of Maryland Snakes by Bird Species
 Herbert S. Harris, Jr.58

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Geographic Variation in Northern Green Frog Larvae, *Lithobates clamitans melanotus*, in Northwestern New Jersey

Johu K. Koroky* & John A. Smallwood

Abstract

A total of 124 larvae of the northern green frog, *Lithobates clamitans melanotus* (Rafinesque 1820), were collected at five localities in three adjacent counties of northwestern New Jersey from 2001 to 2007. Data were recorded for 19 varying character states that included 18 morphometric features (body dimensions and characteristics of the oral disc) and developmental stage. Developmental stage differed significantly among the localities. Tables of univariate descriptive statistics are provided for the 18 morphological features from all sites. Regression analyses of body length over developmental stage and tail length over developmental stage determined that larvae from one locality (Allamuchy State Park) differed markedly from the larvae from the other four localities. Four localities expressed the larval tooth row formula (LTRF) 2(2)/ 3, while Chubb Park was 2(2)/ 3(1). Phenotypic plasticity likely accounts for some of the variation of all characters.

Introduction

Northern green frogs are commonly found throughout New Jersey in a variety of permanent, freshwater habitats, and are named *Rana clamitans melanota* by Schwartz and Golden (2002). Using mtDNA data, Hillis and Wilcox (2005) retained the use of the genus *Rana*, placing them in their Aquarana group. Based on molecular data, Frost et al. (2006) placed the species *clamitans* in the genus *Lithobates*, requiring the use of this genus with the subspecific name changing to *melanotus*, but retaining the common name as green frog. In contrast, Austin and Zamudio (2008) presented mtDNA data that suggested the recognition of the subspecies was not supported. However, their finding was rejected by an eminent anuran systematist group (Center for North American Herpetology, <http://cnah.org/detail.asp?id=1163>). As a result, Collins and Taggart (2009) designated the green frog as *Lithobates clamitans melanotus* (Rafinesque 1820), as does Frost (2011).

Since natural selection operates on anuran larvae (tadpoles) as well as adults, studies focused on the larvae are warranted. This study entails the examination of 124 field-collected larvae from five sites in three adjacent counties of northwestern New Jersey (Morris, Warren, Sussex), known as The Highlands. The purpose of the study was to: (1) document the occurrence of the taxon with habitat notes, (2) document geographic variation of 18 morphometric characters from selected sites using descriptive statistics, and (3) analyze patterns of variation among populations.

Methods

Field collections: Field collections of 124 tadpoles were made by one of us (JKK) at five different sites between 2001 and 2007. Three of the sites were collected twice in two different years. The following are the collection localities and dates of collection. (1) Chubb Park, State Route 24, Chester, Morris County (40° 46' 57.5" N, 74° 42' 36.1" W; elevation 253 m AMSL), a 0.71-ha rectangular, man-made pond of an approximately 1-m uniform depth used for winter ice skating in 34-ha park of grassland and woodlands. A total of 21 specimens were collected on 3 July 2001 and 26 June 2002. (2) Intersection of Colby Farm Road and Knollwood Terrace, Chester, Morris County

Key words: *Lithobates clamitans melanotus*, green frog tadpoles, morphometric variables, oral disc, phenotypic plasticity, New Jersey.

(40° 46' 48.5" N, 74° 40' 58.9" W; elevation 249 m), a 0.05-ha artificial retention basin in a housing subdivision, with marginal aquatic vegetation surrounding a ≥1.25-m deep circular basin. A total of 13 specimens were collected on 3 July 2001 and 26 June 2002. (3) Allamuchy State Park, Deer Park Road, Hackettstown, Warren County (40° 53' 15.0" N, 74° 49' 23.5" W; elevation 273 m), a 0.05-ha natural pond of approximately 1 m depth near a house on the access road to interior of approximately 3500 ha of diverse habitat. Twenty-seven specimens were collected on 21 June 2007. (4) Schooley's Mountain, West Springtown Road, Long Valley, Morris County (40° 47' 6.0" N, 74° 48' 29.4" W; elevation 329 m), a 1.50-ha spring fed pond, approximately 3.5 m deep at center on residential property. Thirty-three specimens were collected on 11 September 2006. (5) New Jersey School of Conservation, Montclair State University, Branchville, Sussex County (41° 13' 1.7" N, 74° 44' 50.2" W; elevation 268 m), a 0.2-ha flooded beaver pond area with slowly moving water and fallen trees. A total of 30 specimens were collected on 8 July 2002 and 14 July 2003.

TABLE 1. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Chubb Park, Morris County, New Jersey, 2001-2002. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 ($n = 3$), stage 28 ($n = 2$), stage 30 ($n = 2$), stage 36 ($n = 3$), stage 37 ($n = 1$), stage 38 ($n = 2$), stage 39 ($n = 2$), stage 40 ($n = 4$), and stage 41 ($n = 2$).

Variable	<i>n</i>	Mean	Median	SD	Range
Body length	21	29.3	33.0	9.1	13.0-39.0
Tail length	21	42.7	48.0	13.9	18.0-59.0
Total length	21	72.0	80.0	23.0	31.0-97.0
Tail height	21	14.6	16.0	3.7	7.0-19.5
Tail muscle height	21	7.5	8.1	2.3	3.5-10.0
Dorsal fin height	21	4.7	4.8	1.2	2.5-7.0
Ventral fin height	21	3.5	3.5	0.9	2.0-5.2
Interocular distance	21	10.0	11.0	3.8	3.0-14.5
Internareal distance	21	3.4	3.5	0.9	1.5-4.6
A-1 length	21	3.6	3.7	1.5	0.7-6.0
Left A-2 length	12	0.9	0.9	0.4	0.3-1.4
Right A-2 length	14	1.0	1.0	0.4	0.5-1.7
A-2 gap	12	2.5	2.5	0.6	1.5-3.6
A-2 gap ratio	12	0.5	0.5	0.2	0.2-0.9
P-1 length	21	3.5	3.6	1.2	1.1-4.8
P-1 gap	6	0.2	0.2	0.1	0.1-0.3
P-2 length	21	3.4	3.5	1.1	0.9-4.6
P-3 length	14	2.4	2.4	1.1	0.2-3.7

Tadpoles were obtained by hand net and preserved in 10% formalin, and are in the custody of the senior author. Larvae were identified by keys (Altig 1970, Altig and Johnston 1986), the online guide of Altig et al. (<http://www.pwrc.usgs.gov/tadpole/>), and the presence of adults in some cases. Larvae were staged according to Gosner (1960).

Morphological measurements: Measurements of body features were made with Cenco calipers, whereas those of the oral disc were made with dissecting microscope and ocular micrometer calibrated to the nearest 0.1 mm. Descriptive features follow Altig (1970) and McDiarmid and Altig (1999), and included body length, tail length, total length, tail height, tail muscle height, dorsal fin height, ventral fin height, interocular distance, internareal distance, A-1 length, left A-2 length, right A-2 length, A-2 gap, A-2 gap ratio, P-1 length, P-1 gap, P-2 length, and P-3 length. Thus,

TABLE 2. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Colby Farm Road, Morris County, New Jersey, 2001-2002. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 ($n = 1$), stage 27 ($n = 1$), stage 28 ($n = 1$), stage 29 ($n = 1$), stage 36 ($n = 1$), stage 37 ($n = 2$), stage 38 ($n = 1$), stage 41 ($n = 1$), stage 42 ($n = 1$), stage 43 ($n = 1$), and stage 44 ($n = 2$).

Variable	<i>n</i>	Mean	Median	SD	Range
Body length	13	24.2	26.0	6.4	13.0-36.0
Tail length	13	33.8	36.0	10.2	20.0-47.0
Total length	13	57.5	60.0	14.3	33.0-74.0
Tail height	13	11.9	11.0	4.6	5.0-19.5
Tail muscle height	13	5.8	5.1	1.9	2.5-8.8
Dorsal fin height	13	4.0	3.5	1.6	1.5-6.5
Ventral fin height	13	2.8	3.0	1.4	1.0-5.4
Interocular distance	13	6.8	6.8	1.8	3.5-9.4
Internareal distance	13	3.1	3.4	0.6	1.8-3.7
A-1 length	10	2.8	3.1	0.9	1.5-3.7
Left A-2 length	3	0.5	0.6	0.2	0.3-0.6
Right A-2 length	4	0.6	0.5	0.4	0.2-1.2
A-2 gap	3	2.0	1.9	0.2	1.8-2.2
A-2 gap ratio	3	0.4	0.3	0.2	0.2- 0.6
P-1 length	10	2.6	2.7	0.7	1.4-3.4
P-2 length	10	2.3	2.6	0.9	1.1-3.5
P-3 length	7	1.6	1.6	0.4	1.0-2.0

16 direct measurements, 2 derived variables (total length and A-2 gap ratio), and developmental stage were recorded for each tadpole from the five selected sites. Some specimens had missing or damaged body or oral disc features.

Data analyses: We calculated descriptive statistics of central tendency and variability for each of the 18 morphometric variables. However, developmental stage differed significantly among the five locations (Kruskal-Wallis Rank Sums Test, chi-square approximation = 42.6, $df = 4$, $P < 0.0001$). Thus, we did not compare these variables directly among the five locations. Instead, following Strauss and Altig (1992) we first converted measurements to natural logarithms, and then for each location we used regression models to describe the change in selected morphometric variables in relation to developmental stage. We then compared those regressions among locations. Tests of significance were performed using JMP version 8.0.2, and regression models were calculated with TableCurve 2D version 5.01.

TABLE 3. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Allamuchy State Park, Warren County, New Jersey, 2007. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 31 ($n = 1$), stage 32 ($n = 5$), stage 33 ($n = 9$), stage 34 ($n = 5$), stage 35 ($n = 5$), and stage 36 ($n = 2$).

Variable	<i>n</i>	Mean	Median	SD	Range
Body length	27	9.6	10.0	0.9	7.5-10.5
Tail length	27	10.6	11.0	1.6	8.0-13.5
Total length	27	20.2	21.0	2.2	15.5-24.0
Tail height	27	3.7	3.5	0.5	2.8-4.5
Tail muscle height	27	1.8	2.0	0.3	1.2-2.2
Dorsal fin height	27	1.3	1.2	0.3	0.5-2.0
Ventral fin height	27	1.4	1.4	0.3	1.0-2.0
Interocular distance	27	2.4	2.4	0.2	2.0-2.8
Internareal distance	27	1.5	1.5	0.2	1.0-1.8
A-1 length	27	1.6	1.6	0.2	0.9-2.0
Left A-2 length	27	0.5	0.5	0.2	0.2-0.8
Right A-2 length	27	0.6	0.6	0.2	0.3-0.8
A-2 gap	27	0.4	0.3	0.1	0.1-0.7
A-2 gap ratio	26	1.7	1.7	0.7	0.6-3.5
P-1 length	27	1.4	1.5	0.2	1.1-1.7
P-2 length	27	1.3	1.3	0.2	1.1-1.7
P-3 length	26	1.1	1.0	0.2	0.8-1.5

Results

Measures of central tendency and variability in the 18 morphometric characters of the samples collected from Chubb Park are presented in Table 1; 6 of 21 larvae had the unique LTRF of 2(2)/3(1), indicating a P-1 median gap. P-1 gap was not expressed on any tadpoles from the other four localities; these larvae showed a LTRF of 2(2)/3. Measures of central tendency and variability in the remaining 17 morphometric characters of specimens from the four other collection localities are presented in Tables 2 through 5.

The following regression model most consistently provided the best fit for the relationship of body length with developmental stage: length = a + b/stage². The samples from Allamuchy State Park differed markedly from the other four locations (Figure 1). The same regression model also most

TABLE 4. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from Schooley's Mountain, Morris County, New Jersey, 2006. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 26 ($n = 1$), stage 27 ($n = 2$), stage 28 ($n = 2$), stage 29 ($n = 4$), stage 30 ($n = 4$), stage 31 ($n = 15$), stage 32 ($n = 1$), stage 33 ($n = 2$), and stage 36 ($n = 2$).

Variable	<i>n</i>	Mean	Median	SD	Range
Body length	33	19.0	19.0	2.4	14.0-24.0
Tail length	33	26.6	27.0	4.1	18.0-35.0
Total length	33	45.6	47.0	6.3	32.0-59.0
Tail height	33	9.7	10.0	1.3	7.0-12.2
Tail muscle height	33	4.1	4.2	0.5	3.2-5.0
Dorsal fin height	33	3.4	3.3	0.5	2.5-4.5
Ventral fin height	33	2.8	2.8	0.4	2.0-3.5
Interocular distance	33	5.3	5.5	0.9	3.8-7.2
Internareal distance	33	3.1	3.2	0.4	2.2-3.9
A-1 length	33	2.3	2.4	0.3	1.6-3.1
Left A-2 length	31	0.3	0.3	0.2	0.1-0.6
Right A-2 length	30	0.4	0.4	0.2	0.1- 0.7
A-2 gap	29	1.2	1.2	0.3	0.9-2.1
A-2 gap ratio	29	0.4	0.4	0.2	0.1-0.7
P-1 length	33	2.1	2.1	0.4	1.1-3.0
P-2 length	33	2.1	2.1	0.4	1.4-3.0
P-3 length	32	1.2	1.2	0.4	0.6-2.1

consistently provided the best fit for the relationship of tail length with developmental stage, and again the samples from Allamuchy State Park were markedly different from the samples obtained at the other four locations (Figure 2). Mean total length of the Allamuchy State Park larvae (20.2 mm) was less than half the mean lengths from the four other localities (45.6-72.0 mm; Tables 1-5).

Discussion

Altig and Johnston (1989, Table 1) showed the LTRF 2/3 to be most common (51%) and highly conserved of 320 anuran species studied of a total of 627. All our 124 larvae were slight derivatives of this prime formula, and are consistent with the species being a lentic-benthic pond form with reduced oral apparatus complexity.

TABLE 5. Descriptive statistics of selected character states for *Lithobates clamitans melanotus* larvae from the School of Conservation, Sussex County, New Jersey, 2002-2003. All measurements are in mm. Range is minimum value-maximum value. Developmental stages for specimens included stage 28 ($n = 2$), stage 29 ($n = 1$), stage 32 ($n = 1$), stage 35 ($n = 1$), stage 36 ($n = 4$), stage 37 ($n = 2$), stage 38 ($n = 1$), stage 39 ($n = 6$), stage 40 ($n = 4$), stage 41 ($n = 7$), stage 42 ($n = 1$), and stage 46 ($n = 1$).

Variable	<i>n</i>	Mean	Median	SD	Range
Body length	30	26.9	27.5	3.3	17.0-33.0
Tail length	30	41.7	43.0	8.9	21.0-59.0
Total length	30	68.6	70.0	11.6	39.0-88.0
Tail height	30	13.9	14.5	2.4	7.5-19.0
Tail muscle height	30	7.6	7.7	1.3	4.0-9.5
Dorsal fin height	30	4.2	4.5	0.7	2.5-5.5
Ventral fin height	30	2.8	2.9	0.8	1.0-4.5
Interocular distance	28	8.5	8.8	0.7	6.5-9.6
Internareal distance	30	3.8	4.0	0.4	3.0-4.4
A-1 length	27	2.5	2.8	0.7	0.5-3.3
Left A-2 length	10	0.3	0.4	0.1	0.1-0.6
Right A-2 length	8	0.5	0.5	0.3	0.2-1.1
A-2 gap	4	2.1	2.0	0.5	1.8-2.8
A-2 gap ratio	4	0.3	0.3	0.1	0.2-0.3
P-1 length	27	2.7	2.7	0.5	1.3-3.8
P-2 length	27	2.2	2.3	0.5	1.0-2.8
P-3 length	21	1.1	1.1	0.5	0.2-2.0

While developmental stage was determined to be statistically different between localities, variability in the oral apparatus cannot be explained solely by stage difference as the assumption that stage and oral disc development are tightly correlated is not warranted McDiarmid and Altig (1999, p.45).

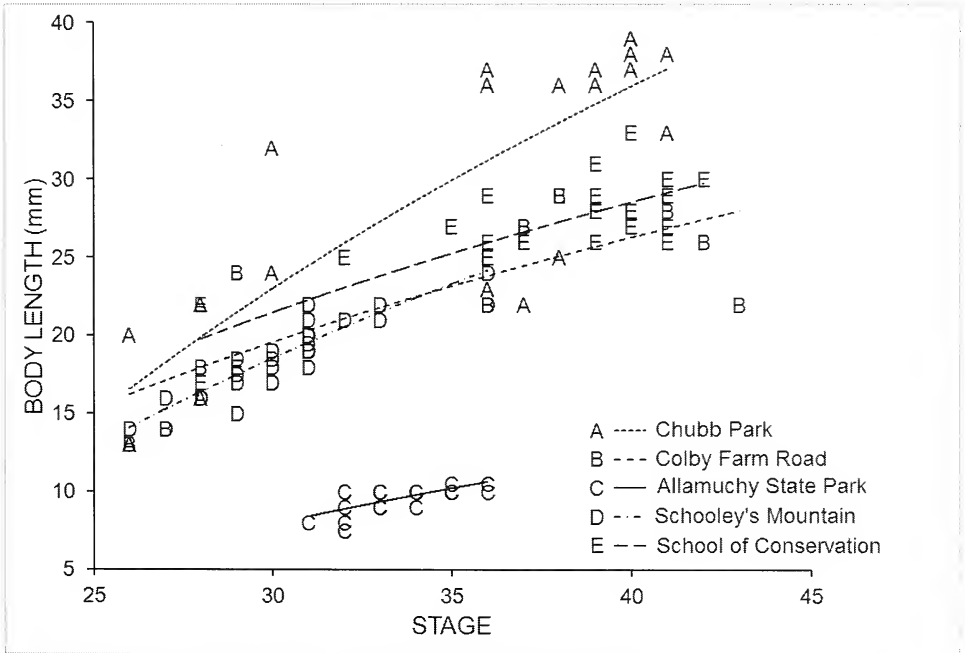
It is notable the two localities in Chester, Chubb Park and Colby Farm Road, are about 2.5 km apart. Yet, the former had 6 of 21 specimens with a P-1 gap, while none of the 13 at the latter had any with that gap. The specific reason for this is unclear.

Variation in the data may be attributed to some combination/permutation of the following factors: ontogenetic variation, nonadaptive variation, and phenotypic plasticity. The percentage each factor may contribute singularly or synergistically has not been determined in studies, but phenotypic plasticity has been investigated the most, particularly with tadpoles as subjects.

Phenotypic plasticity is an adaptive phenomenon wherein one genotype can produce multiple phenotypes as a function of abiotic and biotic factors. This plasticity can result in behavioral, physiological, morphological, and life history alterations (Miner et al. 2005).

The nature of the habitat alone, field-collected versus laboratory-reared, was shown by Hillis (1982) to induce morphological variation in conspecifics. Not surprisingly, food availability affected both age and size at metamorphosis (Hensley 1993). Impending pond desiccation increased

FIGURE 1. The relationship of body length and developmental stage for *Lithobates clamitans melanotus* larvae collected at five locations in northwestern New Jersey, 2001-2007. Regression model for Chubb Park: $\ln(\text{length}) = 4.1 - 949.3/\text{stage}^2$, $r^2 = 0.73$; for Colby Farm Road: $\ln(\text{length}) = 3.7 - 668.7/\text{stage}^2$, $r^2 = 0.71$; for Allamuchy State Park: $\ln(\text{length}) = 3.1 - 951.9/\text{stage}^2$, $r^2 = 0.46$; for Schooley's Mountain: $\ln(\text{length}) = 3.8 - 789.3/\text{stage}^2$, $r^2 = 0.82$; and for School of Conservation: $\ln(\text{length}) = 3.7 - 611.9/\text{stage}^2$, $r^2 = 0.74$.



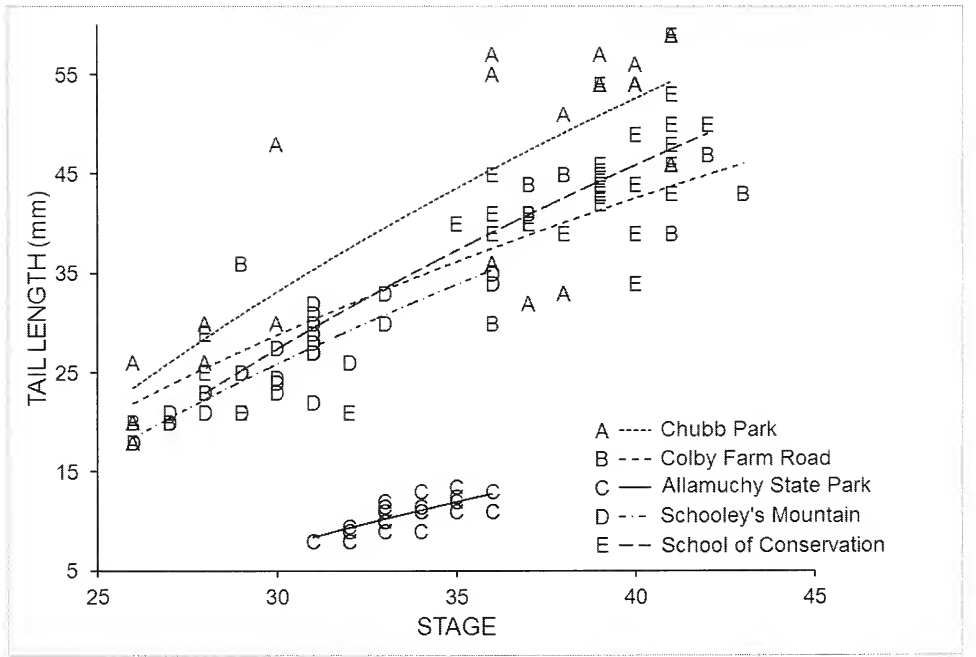
speed of development with earlier metamorphosis and smaller size (Laurila and Kujasalo 1999), an obvious survival facilitator. Predator presence is another factor. Miner et al. (2005), Kraft et al. (2006), and Van Buskirk and Relyea (2008) all demonstrated such presence resulted in smaller bodies with larger tail fins, presumably aiding predator escape by better swimming and being able to survive a tail bite compared to a fatal body bite.

Predator presence also may increase the toxic effects of pesticide use (Relyea 2003). Carbaryl is a water soluble pesticide used globally. He tested green frogs as one of six amphibian species exposed to the pesticide and simultaneous predator stress. The lethality of exposure increased many times with predator presence.

Sometimes the changing subtle interaction of factors may lead to study discordance, as in those of green frog tadpoles. Schalk et al. (2002), using a leech as a high risk larval predator, showed delayed metamorphosis and a larger size at metamorphosis. Ireland et al. (2007), using a leech as a green frog egg predator, determined metamorphosis occurred at an earlier stage with a smaller size at hatching. Thus, the same predator at a different life history stage of the same species produced markedly different plasticity outcomes.

Suffice to say, further investigations of the abiotic and biotic factor interplay influencing larval morphology are warranted, and will be challenging evolutionary biology, but will better illuminate the mechanisms of Natural Selection.

FIGURE 2. The relationship of tail length and developmental stage for *Lithobates clamitans melanotus* larvae collected at five locations in northwestern New Jersey, 2001-2007. Regression model for Chubb Park: $\ln(\text{length}) = 4.6 - 983.7/\text{stage}^2$, $r^2 = 0.73$; for Colby Farm Road: $\ln(\text{length}) = 4.3 - 843.1/\text{stage}^2$, $r^2 = 0.80$; for Allamuchy State Park: $\ln(\text{length}) = 3.8 - 1630.9/\text{stage}^2$, $r^2 = 0.57$; for Schooley's Mountain: $\ln(\text{length}) = 4.3 - 926.8/\text{stage}^2$, $r^2 = 0.77$; and for School of Conservation: $\ln(\text{length}) = 4.5 - 1052.0/\text{stage}^2$, $r^2 = 0.69$.



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Seasonal Activity, Reproductive Cycles, and Growth of the Bronze Frog (*Lithobates clamitans clamitans*) at the Western Edge of its Geographic Range

Abstract

Seasonal activity, reproduction, and growth of the Bronze Frog (*Lithobates clamitans clamitans*) from Texas were examined using 278 museum specimens and calling data. Post-metamorphic individuals were active throughout the year, and metamorphosing were captured over an extended season. Males called, and females were gravid, over an extended time in eastern Texas. Larval transformation occurred at small body sizes and sexual maturity was reached quickly and at small body sizes, with males being smaller in mean body size than females. Findings relating to activity, reproduction, and growth in this study were in general agreement with those from Louisiana. Our findings suggest that factors apart from those studied here were responsible for the western limit of its geographic range.

Introduction

The Bronze Frog, *Lithobates clamitans clamitans* (Latreille 1801), is one of two recognized subspecies of the eastern North American Bronze Frog, *L. clamitans* (Latreille 1801). Occurring in the southeastern United States, it intergrades with the Green Frog, *L. clamitans melanotus* (Rafinesque 1820) along the fall line in Georgia and Alabama, which in turn replaces the Bronze Frog north to southeastern Canada (Conant and Collins 1998; Pauley and Lannoo 2005). Less attention has been paid to the Bronze Frog in the literature than its nearest relative despite the ubiquity of this species in generally lentic aquatic systems in the southeastern United States. Examination of this species in Louisiana (Meshaka et al. 2009a,b) corroborated findings of small body size of metamorphosing (Wright and Wright 1949) and adults (Wright and Wright 1949; Mecham 1954) of the Bronze Frog and found longer seasons of activity and reproduction and faster post-metamorphic growth to sexual maturity than in northern populations of the Green Frog (Meshaka et al. 2009a). The goal of this study was to compare these same parameters from the western edge of the Bronze Frog's geographic range and the southwestern edge of the Green Frog's geographic range to test the endpoint in the geographic variation of these life history traits

Materials and Methods

Two hundred and seventy-eight specimens of Bronze Frogs (*Lithobates clamitans clamitans*) collected during 1931–2000 from eastern Texas (Figure 1) were examined from the holdings of the California Academy of Sciences, Carnegie Museum of Natural History, Field Museum of Natural History, Los Angeles County Museum of Natural History, Illinois Natural History Survey, Northwestern State University, Texas Cooperative Wildlife Collection, Texas Memorial Museum, Tulane University, University of Arizona, University of Kansas Biodiversity Institute, University of Michigan, and the University of Texas–El Paso (Appendix 1). Body lengths of all size-classes and of tadpoles were measured in mm snout-vent length (mm SVL).

Sexual maturity was determined in males using a slightly modified version of the technique by Martof (1956), whereby the ratio of tympanum diameter: body size corresponded to enlarged

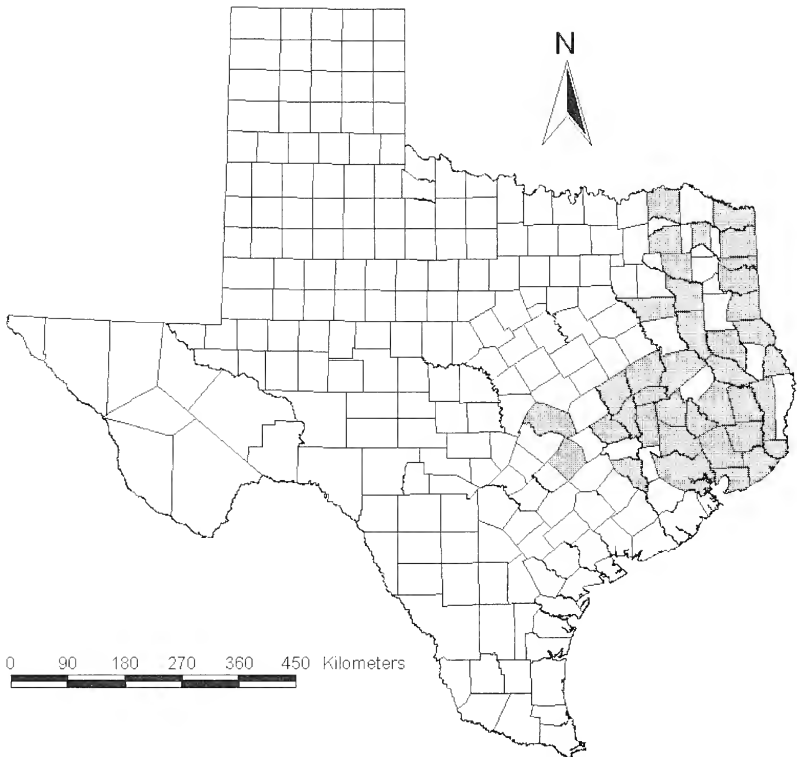
Keywords. Bronze Frog, ecology, frogs, life history,

testis, which signified sexual maturity. Martof (1956) noted that the tympana generally were “nearly or quite round.” For most frogs Martof (1956) measured the antero-posterior diameter of the left tympanum. If irregular in shape, the right tympanum was measured, and if both were misshapen, Martof (1956) took the average of the antero-posterior and dorso-ventral measurements. Irregularly shaped tympana from our sample were greater in length than in height. For consistency, the dorso-ventral diameter of the left tympanum was measured, and the right tympanum was measured only if the left one appeared to have been damaged in some way. As per Martof (1956), sex index = body length/ tympanum diameter. The sex index was generally below 10 for sexually mature males (Martof 1956).

The secondary sexual characteristic of enlarged thumbs was not easily ascertained. The yellow throat of mature males, which easily fades to varying degrees in preservative, was not apparent. The length and width of the left testis as a percent of the body size was used to measure seasonal differences in testis dimensions.

Sexually mature females were associated with one of four ovarian stages. In the first ovarian stage oviducts were thin and just beginning to coil, and the ovaries are somewhat opaque. In the second ovarian stage, the oviducts were larger and more coiled, and the ovaries contained some pigmented oocytes. In the third ovarian stage, oviducts were thick and heavily coiled, and the ovaries were in various stages of clutch development. In the fourth ovarian stage, oviducts were

Figure 1. Texas counties from which 278 museum specimens of Bronze Frogs (*Lithobates clamitans clamitans*) were examined in this study.



thick and heavily coiled, and the ovaries were full of polarized ova with few non-polarized ova, signifying a fully ripened clutch and gravid female (Meshaka 2001). Fat body development was scored as absent, intermediate in volume in the body cavity, to extensive development that reached upwards in the body cavity. The latter amount was used as an estimation monthly incidence of extensive fat relative to all females examined in each month.

Tadpoles were scored as per Gosner (1960). For practical purposes, tadpoles were in categories of having poorly-developed hind legs (less than Gosner stage 37) or well-developed hind legs (Gosner stage of at least 37). Metamorphosing tadpoles were distinguished from tadpoles by the presence of forelimbs (Gosner stage 42) and distinguished from juveniles by the presence of a tail. Statistical analysis was conducted with the use of Excel. Means were followed by ± 2 standard deviations, and significance was recognized at $P < 0.05$.

The following sources of calling records were shared in response to Research Request #1 from the Center of North American Herpetology. Calling was monitored at Ratcliff Lake in the Davy Crockett National Forest (DCNF) in Houston County of eastern Texas during 1961–1982 (Edward Greding, unpubl. data). Also at DCNF, calling was monitored at four ponds during 2000–2004 (Daniel Saenz unpubl. data). Calling was monitored at four sites at Brazos Bend State Park (BBSP), Fort Bend County, of eastern Texas during January 1999–November 2004 by DH. Exceptionally, July and October 2004 were not monitored. Those data are presented as number of sites with calls. Regional calling data from the Texas Amphibian Watch and FrogWatch USA volunteers and the U.S. Forest Service, Southern Forest Experimental Station during 1999–2003 was provided by Lee Ann Johnson Linam of the Texas Parks and Wildlife Department. Regions corresponded to North American Amphibian Monitoring Program sampling regions.

Results

Seasonal activity.— Bronze Frogs from southern Texas were collected in every month of the year (Figure 2). Most individuals were found during April–June, especially males and this seasonal peak was followed by a November peak comprised mostly of juveniles (Figure 2).

Seasonal changes in testis size.— Measured as a percentage of male body size, testis length and width were largest in Spring (Figure 3).

Calling.— Bronze Frogs were heard calling during 22 April–10 August at Ratcliff Lake in DCNF. At four ponds also at DCNF, calling began in March ($n = 8$) (earliest = 17 March 2004) or April ($n = 7$) and ended in August ($n = 3$) or September ($n = 12$) (latest = 10 September 2000). At BBSP calling was heard during March–September (Figure 4). Regional monitoring revealed calling during March–October in coastal Texas and during March–September in north/east Texas.

Air (mean = 24.4 ± 2.6 °C; range = 18–29; $n = 25$) and water (mean = 24.5 ± 2.7 °C; range = 18–28; $n = 22$) temperatures associated with calling at BBSP were generally warm, most having been within 24–27 °C range (Figure 5). Relative humidity associated with calling was high, generally 80–90% RH (Figure 6). Calling also occurred in primarily still conditions: < 1 mph ($n = 21$), 1–3 mph ($n = 2$), 4–7 mph ($n = 1$).

Ovarian cycle.— Gravid (stage 4) females were detected during April–September (Figure 7). Yolking-nearly gravid (stage 3) females were captured during March–August (Figure 7). The high frequency of stage 3 females in March, and the highest frequencies of stage 1 and 2 females during October–December were suggestive of a gravid condition having occurred during March–September (Figure 7).

Female fat cycle and the presence of food.— The extent to which fat bodies were well-

Figure 2. Seasonal incidence of captures of 272 Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

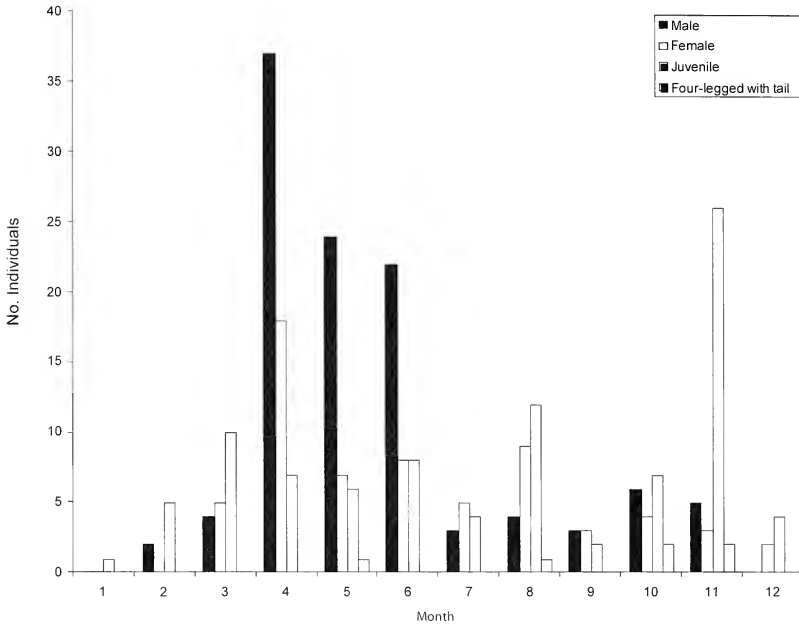


Figure 3. Monthly distribution of testis size as a percentage of body size of 100 Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

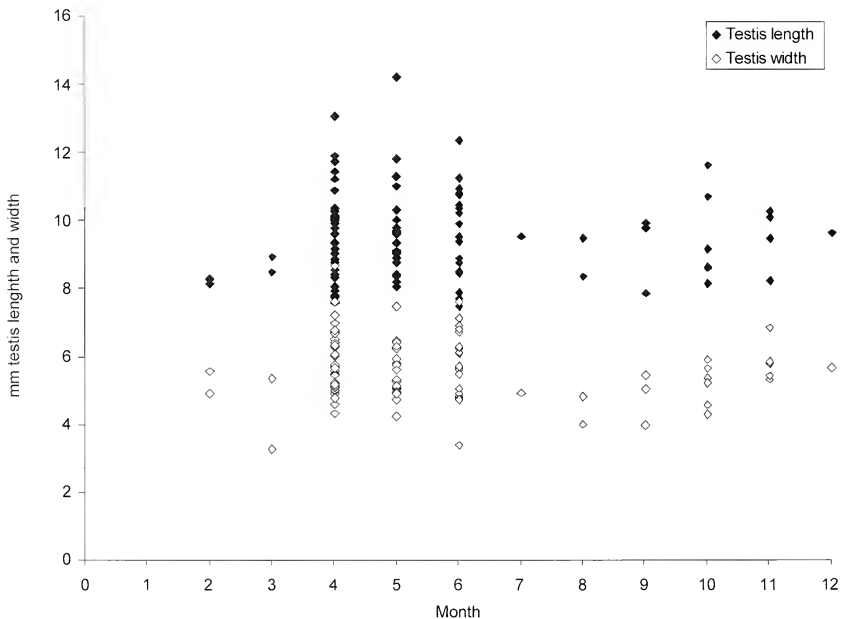


Figure 4. Monthly distribution of 25 calling records for Bronze Frogs (*Lithobates clamitans clamitans*) from Brazos Bend State Park, Fort Bend County, Texas, during 1999-2004.

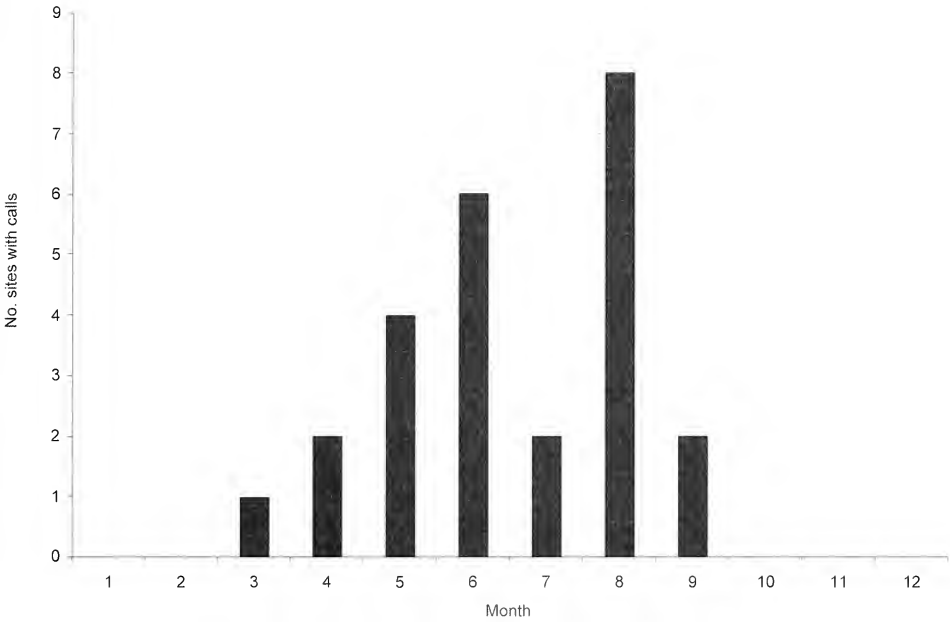


Figure 5. The distribution of air (n = 25) and water (n = 22) temperatures associated with calling by the Bronze Frog (*Lithobates clamitans clamitans*) from Brazos Bend State Park, Fort Bend County, Texas, during 1999-2004.

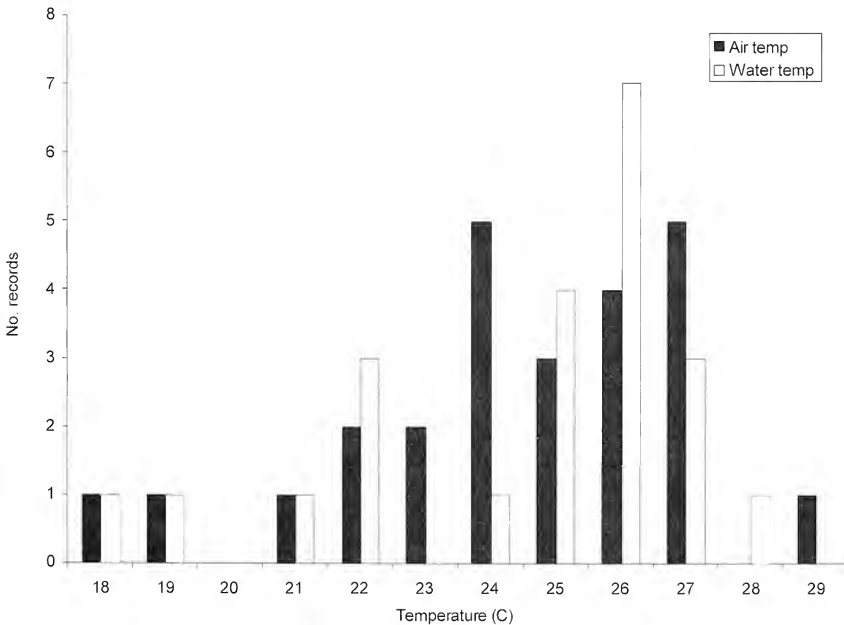
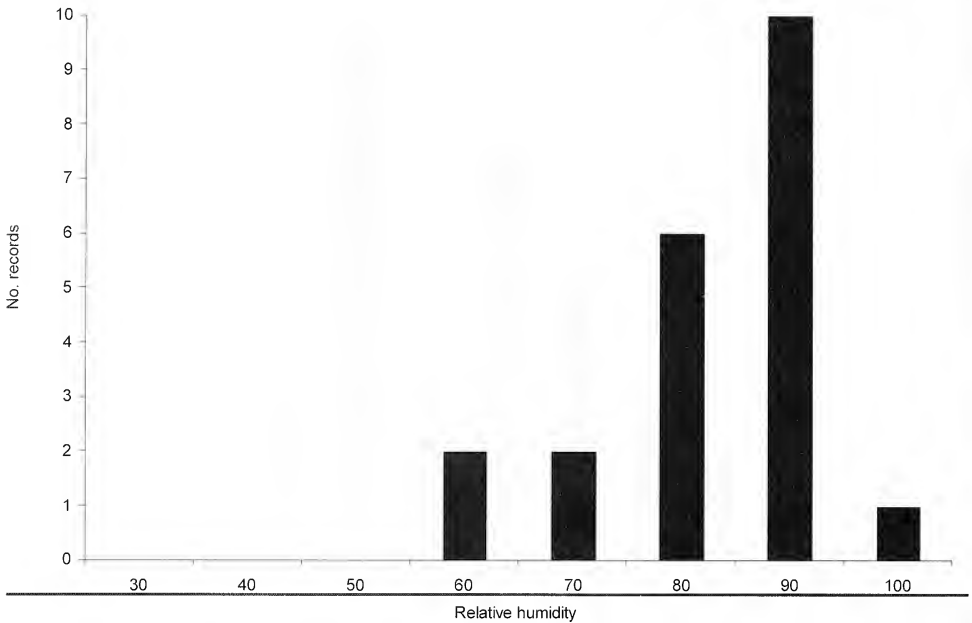


Figure 6. The distribution of relative humidity values ($n = 24$) associated with calling by the Bronze Frog (*Lithobates clamitans clamitans*) from Brazos Bend State Park, Fort Bend County, Texas, during 1999-2004.



developed in females varied across the months, whereby depletion of late-fall stores of fat was evident in the spring and depleted by June (Figure 8). It was during April–June that the highest numbers of gravid females were apparent (Figure 7), the majority of which were depleted of their fat compared to their non-gravid counterparts (Figure 9).

The incidence of females containing food in their stomachs was relatively high through the year but generally highest during September–March (Figure 8). The incidence of females containing prey was lowest during April–August, concomitant with gravid females, the 41.7% of which were not eating (Figure 9).

Growth and sexual maturity.— The length of the larval period in eastern Texas could not be ascertained; however, metamorphoslings were present in May and during August–November (Figure 2, 10), and the distribution of body sizes was suggestive of a nearly continuous production of metamorphoslings in eastern Texas (Figure 10).

Body size at transformation of six metamorphoslings was small (mean = 22.2 ± 3.4 mm SVL) and ranged 18.6–27.2 mm SVL. From these data, growth trajectories from the monthly distribution of body size indicated that male Bronze Frogs in eastern Texas reached sexual maturity in four months of post-metamorphic age at 44.0 mm SVL (Figure 10). Males attained their mean body size three or four months after reaching sexual maturity at 63.0 ± 7.0 mm SVL; range = 44.0–80.7; $n = 112$).

Mean sex index (body length/tympanum) for 111 male Bronze Frogs was 7.4 ± 0.62 mm (range = 6.1–8.7). Tympanum diameter co-varied with the body size of adult males (Figure 11)

Figure 7. The annual ovarian cycle of 52 Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

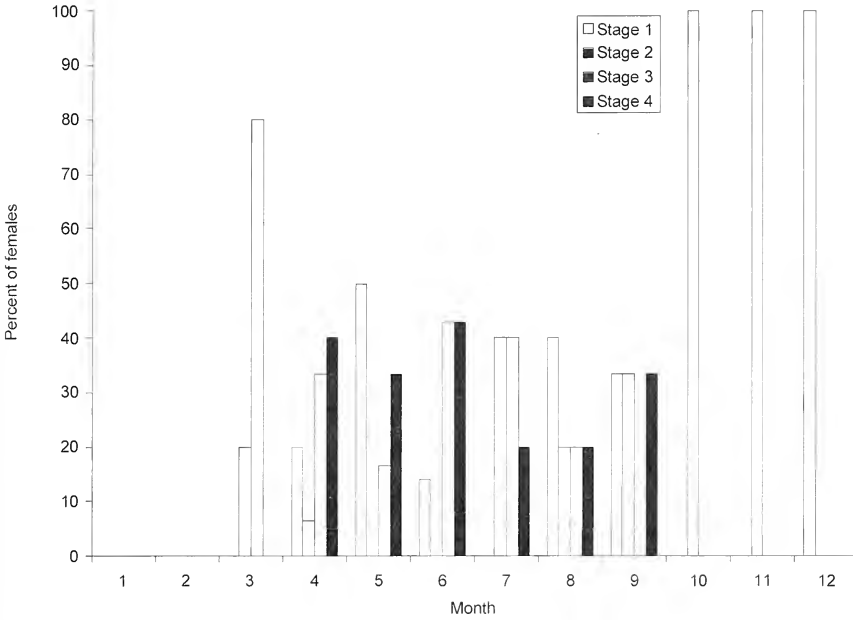


Figure 8. Monthly frequency of extensive fat (n = 48) and the presence of food (n = 38) in female Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

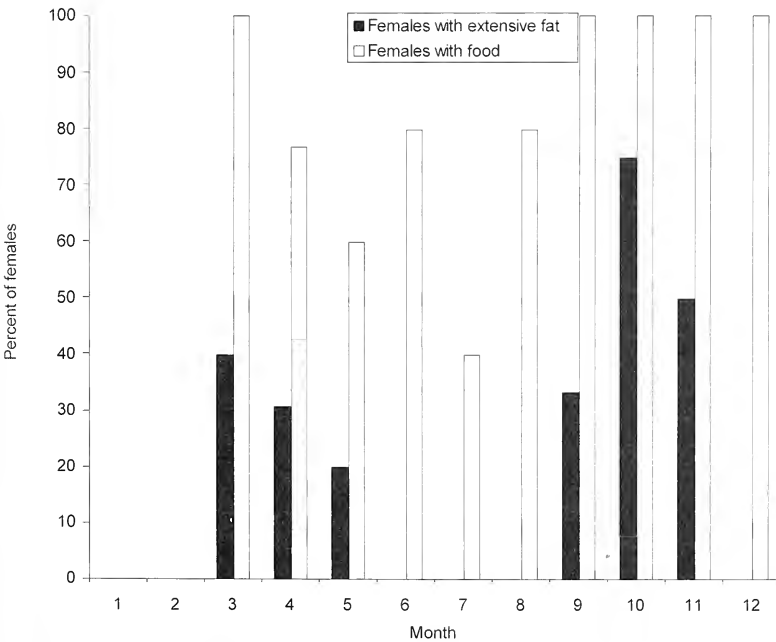


Figure 9. Frequency of extensive fat (n = 48) and the presence of food (n = 48) in each of the four ovarian stages of female Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

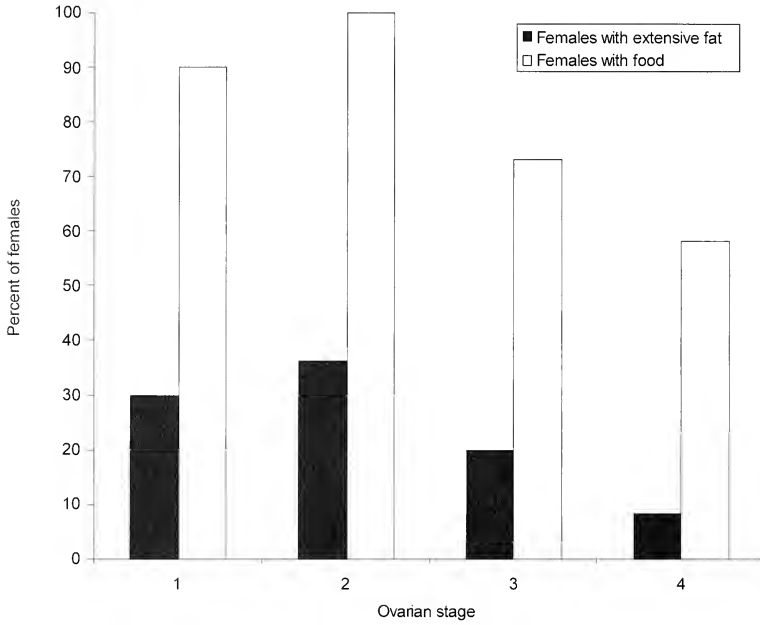


Figure 10. Monthly distribution of body sizes of 272 Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.

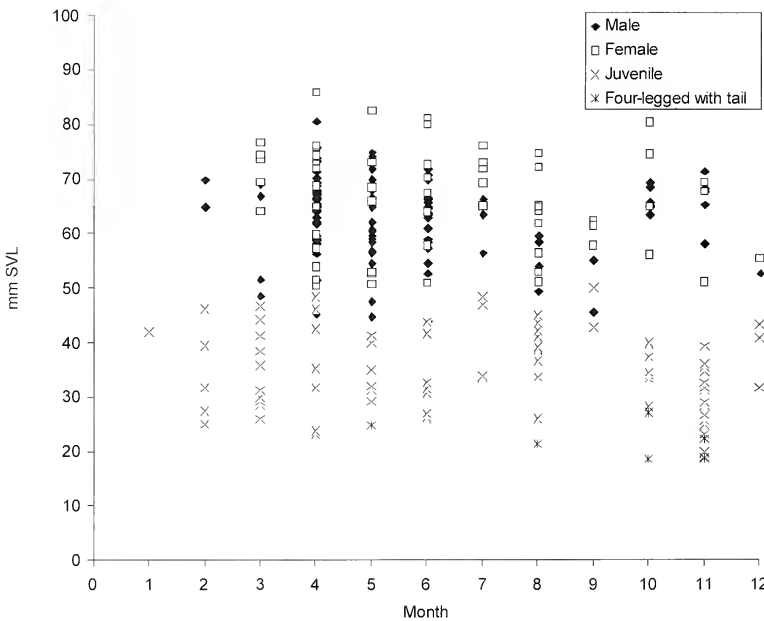
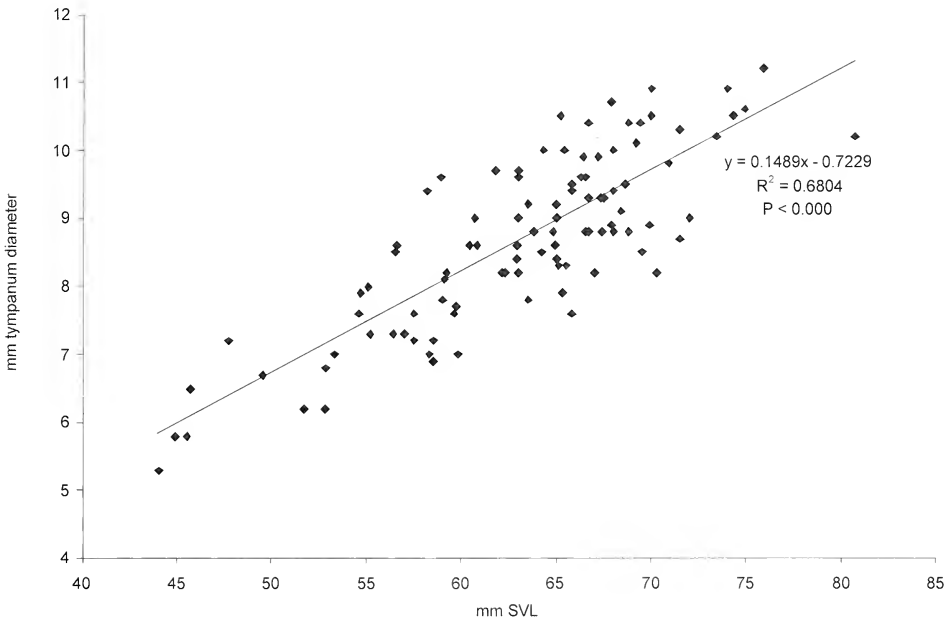


Figure 11. The relationship between tympanum diameter and body size of 111 male Bronze Frogs (*Lithobates clamitans clamitans*) from eastern Texas.



but not strongly enough that that a significant negative relationship existed between the sex index and male body size.

The smallest sexually mature female (ovarian stage 1) reached sexual maturity at five months of post-metamorphic age at 50.6 mm SVL ($n = 11$) (Figure 10). The smallest gravid female measured 57.3 ($n = 15$), and was smaller than the smallest females of ovarian stages 2 (61.5 mm SVL) and 3 (59.8 mm SVL). Mean body size for all sexually mature females was reached approximately five or six months after reaching sexual maturity at 68.2 ± 8.6 mm SVL (range = 50.6–85.9; $n = 53$) mm SVL and their body sizes differed significantly in variance ($F = 0.676$; $P < 0.04$) and mean ($T = -3.883$; $df = 86$; $P < 0.000$) from those of adult males.

Body sizes of gravid females (mean = 71.7 ± 7.1 mm SVL; range = 57.5–82.5; $n = 15$) differed significantly in mean ($T = -1.908$; $df = 51$; $P < 0.03$) from those of non-gravid females (ovarian stages 1–3) (mean = 66.8 ± 8.8 mm SVL; range = 50.6–85.9; $n = 38$).

Discussion

The Bronze Frog is the southern form of two recognized subspecies of the Green Frog, a geographically widespread North American true frog (Conant and Collins 1998). The two forms differ in color pattern (Mecham 1954) and in the smaller body sizes of adults (Wright and Wright, 1949; Mecham 1954) and metamorphosinglings (Wright and Wright 1949). Bronze Frogs of northern and southern Louisiana conformed to these findings of diminution of adult and metamorphosing size (Meshaka et al. 2009a,b). Male Bronze Frogs in Louisiana were also smaller at minimum and adult body size adult than females and they matured earlier than did females (Meshaka et al. 2009a,b). To that end, mean body size of both sexes was smaller in the southern part of the state.

The breeding season was longer in Louisiana than it was farther north in the geographic range of the Bronze Frog (Meshaka et al. 2009a,b).

Findings of this study in eastern Texas were similar to those of Louisiana (Meshaka et al. 2009a,b) with respect to a smaller minimum and mean body size in males, an earlier maturity in males, and a longer breeding season than in Bronze Frogs in northern latitudes. Specifically, however, values of these traits in eastern Texas varied in similarity between northern and southern Louisiana. For example, minimum body size of males at sexual maturity was larger in males from eastern Texas (44.0 mm SVL) than in northern (40.7 mm SVL) and southern (39.9 mm SVL) Louisiana. Likewise, minimum body size at sexual maturity in females was largest in eastern Texas (50.6 mm SVL) as compared to northern (45.2 mm SVL) and southern (43.1 mm SVL) Louisiana. The smallest gravid female from eastern Texas (57.3 mm SVL) was slightly smaller than that of northern Louisiana (60.6 mm SVL) but both were larger than that of southern Louisiana (43.1 mm SVL).

The mean body size of eastern Texas males (63.0 mm SVL) was similar to that of northern Louisiana (mean = 61.0 mm SVL) and larger than that of southern Louisiana (mean = 56.8 mm SVL). Likewise, mean female body size in eastern Texas (68.2 mm SVL) was also larger than that of Louisiana but more similar to the value from northern Louisiana (mean = 66.4 mm SVL) than southern Louisiana (mean = 59.7 mm SVL).

Calling season in coastal Texas exceeded that of southern Louisiana by one month in October. Calling in east, central, northern Texas began in March, like northern Louisiana, and ended in September when the last of the Bronze Frogs were leaving breeding ponds in northwestern Louisiana. The number of months in which gravid females were evident in eastern Texas was prolonged but still fewer than either northern or southern Louisiana. However, limitations of a small sample size from eastern Texas could not be ruled out as an explanation for this difference.

Consequently, the Bronze Frog of eastern Texas typified the small body size and longer breeding season of this southern form and with varying degrees of similarity to those of northern and southern Louisiana populations. The overall similarity of those traits between Texas and Louisiana suggests to us that these traits were not altered by the causal factor or factors, perhaps such as the natural edge of the Eastern Deciduous Forest in eastern Texas, responsible for the western edge of its geographic range.

Acknowledgments

This study would not have been possible without the commitment of the aforementioned institutions to collect and preserve amphibians and reptiles or without the willingness and time taken by institutional staff to pack and ship these specimens for study. To that end, we wish to especially extend our gratitude to Harold A. Dundee for his single-handed efforts in packing and shipping an enormous lot of Bronze Frogs from Tulane. In addition, on 8 July 2004, one of the authors (WEM) made the first e-mail research request ever sent out by *The Center for North American Herpetology*. It asked for information on the life history traits of *Lithobates clamitans*. The response was overwhelming and the tremendous amount of data received through the CNAH request has added to the value of this contribution.

Appendix I. Specimens of Bronze Frogs (*Lithobates clamitans clamitans*) examined for this study.

California Academy of Sciences 162741; Carnegie Museum of Natural history 121300; Field Museum of Natural History 74712, 94459, 94460; Illinois Natural History Survey, 378, 379, 380, 381, 382, 383, 397, 398, 399, 523, 664, 665, 666, 667, 670, 673, 680, 12698, 1358, 1799, 18506, 18507, 18508, 28715; Los Angeles County Museum of Natural History 1681, 1682, 1683, 13864, 13865, 13865, 13866, 35039, 35040, 35041, 35042, 35043, 35044, 35048, 65371, 65372, 65373, 65374, 65375, 65376, 65377, 65379, 65380, 65381, 65383, 65384, 65385, 91264, 91265.

106890, 106891, 106892, 106893, 106894, 106895; Northwestern State University 3292, 4334; Texas Cooperative Wildlife Collection 72, 74, 75, 76, 77, 78, 1058, 2800, 2801, 2802, 2803, 2804, 2805, 2806, 2807, 2808, 2809, 2810, 2811, 2814, 2815, 2817, 2818, 2821, 2823, 2824, 2825, 4317, 4318, 4319, 4320, 4321, 4322, 4980, 4981, 4982, 4983, 5046, 5048, 5049, 5050, 5051, 5052, 5053, 5054, 5055, 5956, 5957, 5959, 5960, 5961, 5962, 9061, 9062, 9063; Texas Memorial museum 856, 1357, 1358, 1375, 2209, 2356, 2392, 2393, 2394, 2395, 2396, 2397, 7241, 8640, 8640, 8642, 8651, 9208, 9735, 12177, 12178, 12179, 12180, 12181, 12182, 12183, 12184, 12185, 14097, 14259, 15230, 15314, 18223, 18240, 18242, 18243, 18244, 18245, 18247, 18248, 18250, 18251, 18252, 18254, 18255, 18256, 18257, 18258, 18259, 18260, 20602, 21443, 21446, 22045, 22046, 22047, 22048, 22259, 22347, 22349, 24869, 25540, 25738, 25739, 25740, 29035, 29136, 30822, 30823, 30824, 33169, 33170, 33171, 33172, 36337, 44278, 46320, 52080, 55224, 55401, 55446, 56007; Tulane University 14396, 15636, 15636, 16756, 17323, 22041, 22054, 22059, 22061, 22072, 22073, 30608, 30616, 30617, 30618, 30619, 30620, 30621, 30622, 30646, 30647, 30648, 30649, 30650, 30651, 30652, 30653, 30654, 30655, 30656, 30657, 30658, 30659, 30660, 30677, 30678, 30679, 30707, 30758, 31107, 31108, 31114, 31115, 31116, 31119, 31120, 31131, 31264; University of Arizona 42329; University of Kansas Biodiversity Institute 60615, 60616, 60617, 60618, 60619, 289459, 289515; University of Michigan 70358, 77630, 77630, 77630, 102206, 102206, 105257, 115845, 115845, 115846, 115847, 16771, 116771, 116771, 116772, 116772; University of Texas-El Paso 8580, 12213, 14004.

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Seasonal Activity, Reproductive Cycles, and Growth of the Northern Leopard Frog, *Lithobates pipiens* (Schreber, 1782), From Pennsylvania

Abstract

An examination of 478 museum specimens of the Northern Leopard Frog (*Lithobates pipiens*) collected during 1896-1985 from Pennsylvania provided comparative life history data from the southeastern edge of its geographic range. Activity occurred during March-October, with breeding in the spring. The larval period lasted two to three months, and both sexes reached sexual maturity within one year of transformation at about 50 mm snout-vent length. Sexual maturity was reached before the first year of post-metamorphic age, but all individuals were breeding just before reaching two years of post-metamorphic age. In most respects, reproduction and growth of this species in Pennsylvania were more similar to populations at similar latitudes than more northerly populations. These differences quantify variability in life history traits which in turn are necessary data when formulating region-specific management plans.

Introduction

The Northern Leopard Frog, *Lithobates pipiens* (Shreber, 1782), is a geographically widespread frog primarily in southern Canada and northern regions of the United States (Conant and Collins, 1998). In Pennsylvania, it occurs primarily in the northern and western counties (Hulse et al., 2001; Meshaka and Collins, 2010), which for the species approximates the southeastern edge of its geographic range (Conant and Collins, 1998; Rorabaugh, 2005). The species ranges farther south in the American West (Conant and Collins, 1998; Rorabaugh, 2005). In summary of the literature Rorabaugh (2005) reported oviposition having generally occurred during a short period in the spring, with exceptions in the southwest, clutch sizes having ranged 648-7648 eggs, and tadpoles having transformed within a wide range of body sizes within three to six months following egg deposition. Age at sexual maturity was generally delayed to two to three years, especially in females, with some individuals, especially males having matured, in less than two years of age (Rorabaugh, 2005).

Very few published data are available for the ecology of this frog in Pennsylvania, where it is listed as a species of Greatest Conservation Need (Morris, 2010). In light of its status in Pennsylvania, paucity of life history information in the state, and variation in life history traits over its large geographic range, we undertook this study to provide region-specific information to both better understand the patterns of its geographic variation in its life history traits near the southeastern edge of its geographic range and to provide the sorts of region-specific information necessary in formulating effective management plans for this sensitive Pennsylvania species.

Materials and Methods

We examined 478 specimens of Northern Leopard Frogs (*Lithobates pipiens*) that were collected during 1898-1985 from 24 of the 67 Pennsylvania counties from the holdings of the Carnegie Museum of Natural History in Pittsburgh and the State Museum of Pennsylvania in Harrisburg (Figure 1). The majority of these specimens, 61.1%, were collected from Allegheny County. Body lengths of all frogs and tadpoles were measured in mm snout-vent length (mm SVL) to the nearest 0.1 mm using calipers.

Key words: Anuran breeding, clutch sizes, geographic variation, species management.

Sexual maturity in males was determined by the presence of enlarged testes and enlarged thumbs. Length and width of the left testis as a percent of the body size was used to measure seasonal differences in testis dimensions. Monthly frequencies of enlarged thumbs also served as a measure of seasonal patterns of fertility.

Sexually mature females were associated with one of four ovarian stages. In the first ovarian stage oviducts were thin and just beginning to coil, and the ovaries are somewhat opaque. In the second ovarian stage, the oviducts were larger and more coiled, and the ovaries contained some pigmented oocytes. In the third ovarian stage, oviducts were thick and heavily coiled, and the ovaries were in various stages of clutch development. In the fourth ovarian stage, oviducts were thick and heavily coiled, and the ovaries were full of polarized ova with few non-polarized ova, signifying a fully ripened clutch and gravid female (Meshaka 2001).

Fat body development was scored as absent, intermediate in volume in the body cavity, to extensive development that reached upwards in the body cavity. The latter amount was used as an estimation monthly incidence of extensive fat relative to all females examined in each month.

A subset of females was examined for clutch characteristics. Clutches were removed, patted on paper towel to remove excess moisture, a subset of ova was weighed on an electronic scale to the nearest 0.1 g, and that mass was extrapolated to estimate clutch size. From each clutch, the diameters of 10 ova were measure using an ocular micrometer; the largest ovum was used in comparative relationships with clutch size and female body size.

Tadpoles from were scored as per Gosner (1960). For practical purposes, tadpoles were in categories of having poorly-developed hind legs (less than Gosner stage 37) or well-developed hind legs (Gosner stage of at least 37). Metamorphoslings were distinguished from tadpoles by the presence of forelimbs (Gosner stage 42) and distinguished from juveniles by the presence of a tail. Statistical analysis was conducted with the use of Excel. Because body size differences were expected in our comparisons, one-tailed t-tests were used to compare means between samples. F-tests were calculated to determine significant differences in variance of samples. Significance was recognized at $P < 0.05$. Means were followed by ± 1 standard deviations.

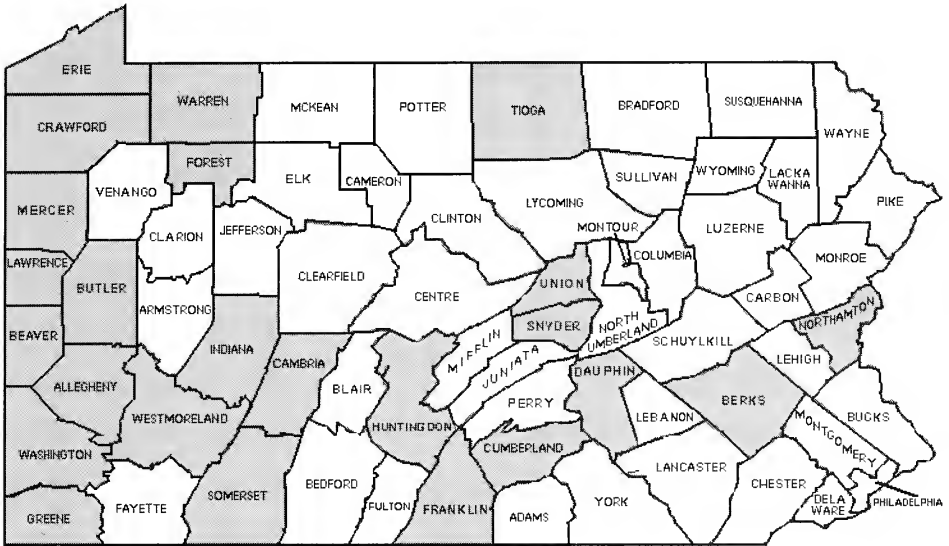
Results

Seasonal activity.- Over a period of 87 years, Northern Leopard Frogs were collected during February-October (Figure 2). Males were most apparent in collections in March and April and less so again in September (Figure 2a). Small samples hampered detection of seasonal trends in numbers of females; however, more females were captured in March than in February, with many more having been detected in September (Figure 2a). Thus, for sexually mature individuals, March, April, and September were peak months in their detectability (Figure 2a). On the other hand, juveniles were most apparent in July and somewhat less so in August, although juveniles were apparent during March-October (Figure 2a). Tadpoles and most metamorphoslings were detected in July (Figure 2b). A few metamorphoslings were detected in June and August (Figure 2b).

Seasonal changes in testis size.- The monthly distribution of testis length as a percentage of snout-vent length was indicative of an apparent decrease from spring into summer, followed by a fall increase in dimensions (Figure 3).

Male thumbs.- In all months except May, enlarged thumbs were present on at least 75% of the males (Figure 4). Enlarged thumbs were detected in all spring-emergent males and those captured at the end of the active season (Figure 4).

Figure 1. Twenty-four of the 67 Pennsylvania counties from which specimens of the Northern Leopard Frog, *Lithobates pipiens*, were examined in this study.



Male fat and presence of food.- The monthly percentage of males with extensive fat development never exceeded 50% of the monthly sample, and both spring and fall samples contained few males with extensive fat development (Figure 5). May samples contained the fewest males containing extensive coelomic fat (Figure 5). The monthly percentage of males containing food was greater in August and September than at other times of the year. Two thirds of males captured near the end of their active season contained food as compared to the earliest collections in March in which only slightly more than one third of the sample had yet eaten since emergence (Figure 5).

Ovarian cycle.- The highest number of gravid females were found in March and April, although some gravid females were found in July and September as well (Figure 6). Females captured at the end of their active season were in various stages of follicular development, including nearly gravid (Figure 6).

Clutch characteristics.- Ten females (mean = 85.4 ± 8.42 mm SVL; range = 66.8-95.0) produced a mean clutch size of 3009.3 eggs (std. dev. = 852.02; range = 1512-4040). The relationship between clutch size and female body size was positive but not significant ($p > 0.05$). The mean ovum diameter of 100 ova from 10 gravid females measured 1.77 mm (std. dev. = 0.35; range = 1.1-2.8). The relationship between mean ovum size female body size was positive and significant ($p < 0.01$) (Figure 7); however, the relationship between mean ovum diameter and clutch size was positive but not significant ($p > 0.05$).

Female fat and presence of food.- The highest incidence of extensive fat among sexually mature females was found in yolking females of stages 2 and 3 (Figure 8). Fully gravid (stage 4) and reproductively quiescent females (stage 1) were least likely to contain extensive fat (Figure 8). The frequency of females containing food declined as follicular development advanced, such that gravid females, whose body cavities were filled with ripe eggs, comprised the segment of females with the lowest incidence of food (Figure 8).

Figure 2. Seasonal incidence of capture of 478 Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania. A = males (n = 54), females (n = 44), and juveniles (n = 204). B = tadpoles with poorly developed rear legs (n = 21), tadpoles with well-developed rear legs (n = 49), and metamorphoslings (n = 106).

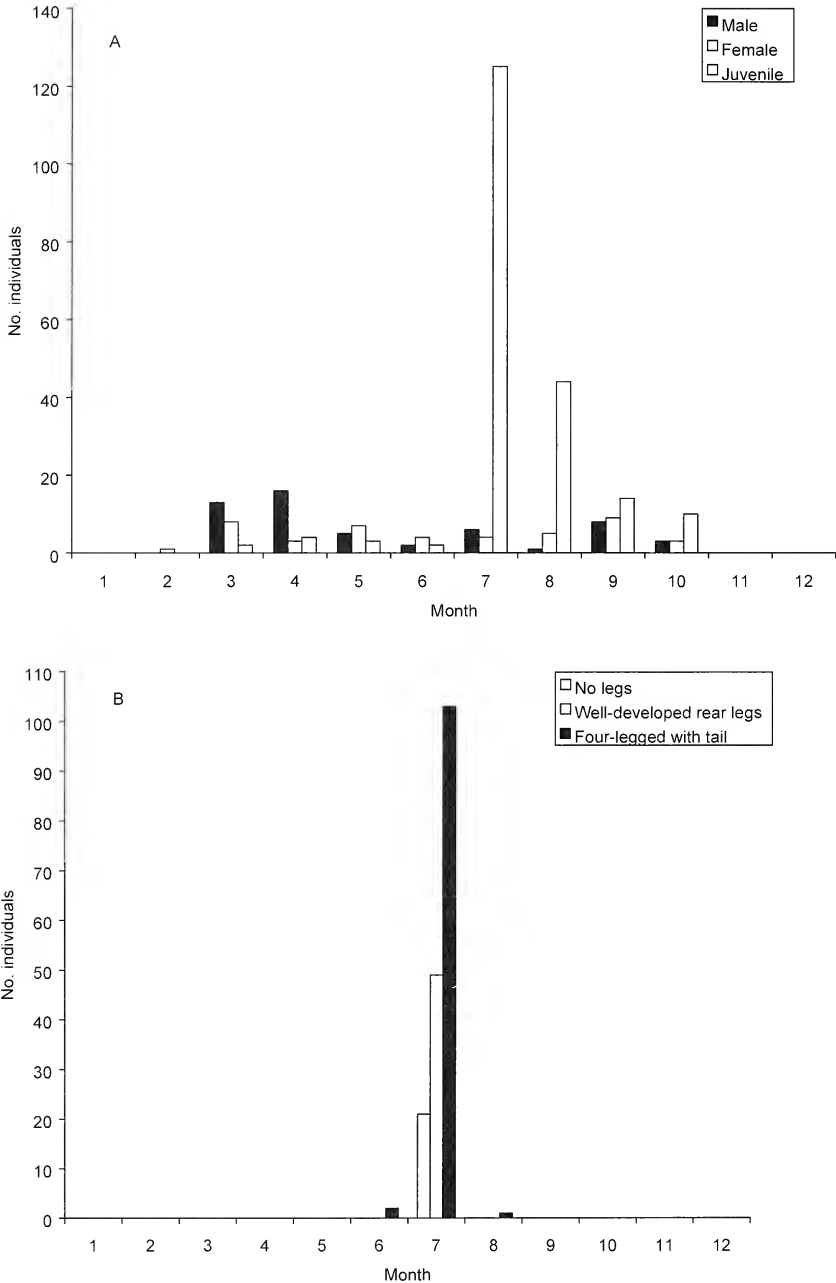


Figure 3. Monthly distribution of testis size as a percentage of snout-vent length of 54 Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

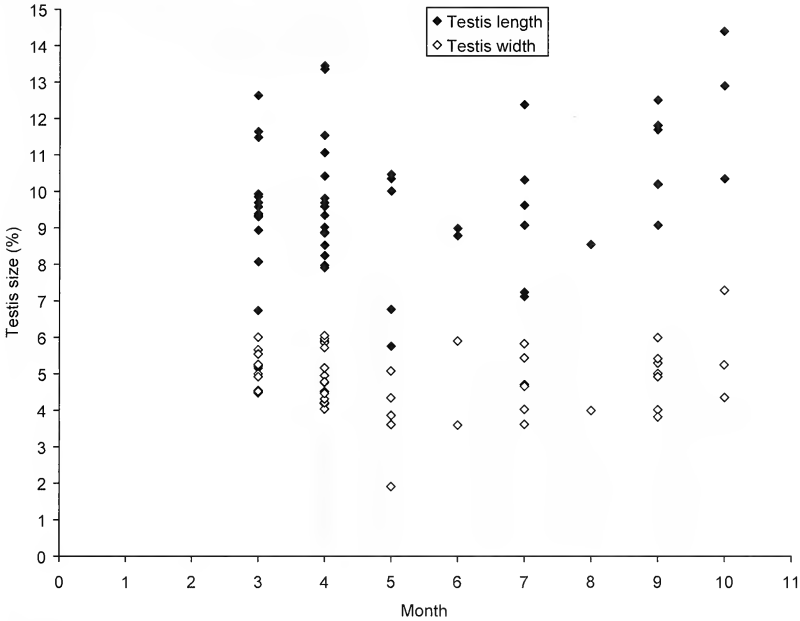


Figure 4. Monthly frequencies of enlarged thumbs in 54 male Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

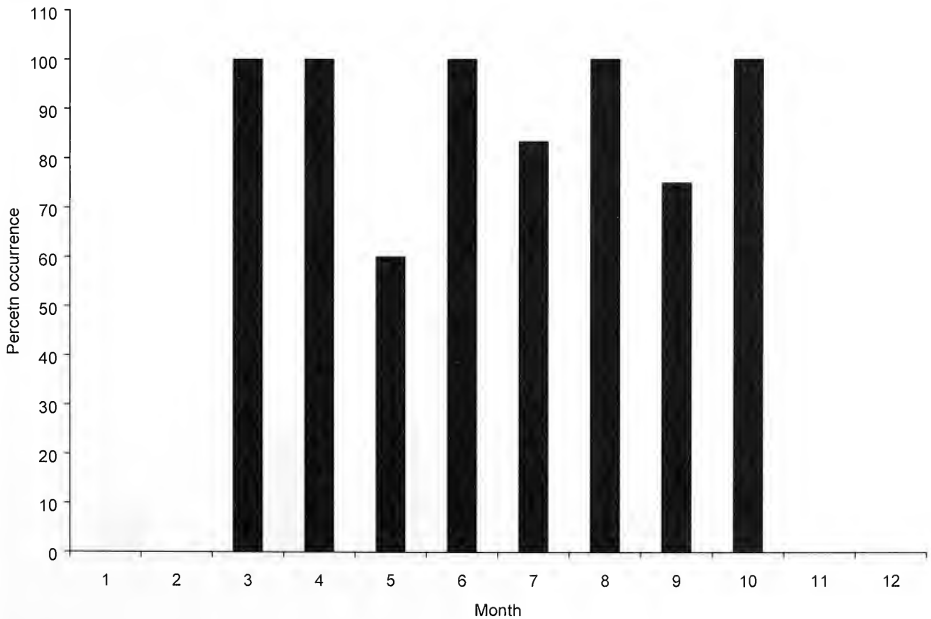


Figure 5. Monthly frequency of extensive fat and the presence of food in 54 male Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

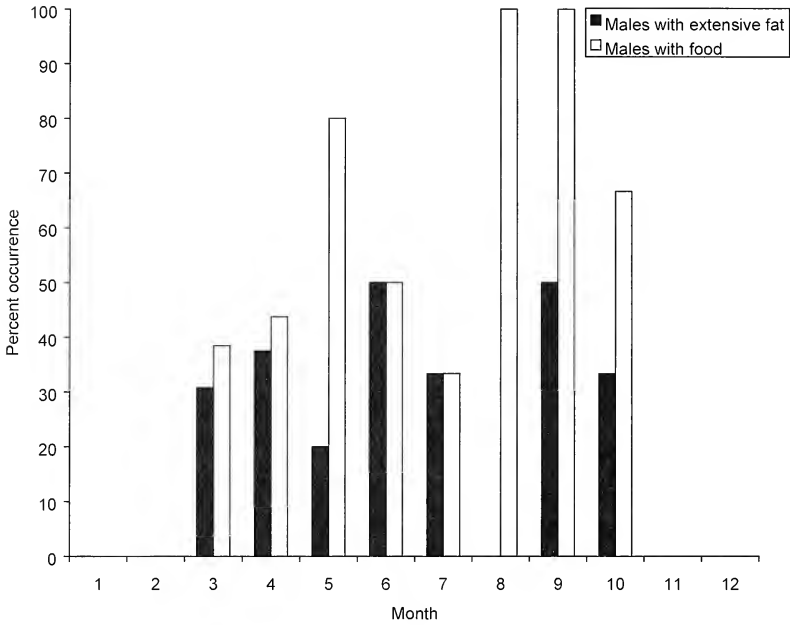


Figure 6. The annual ovarian cycle of 44 Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

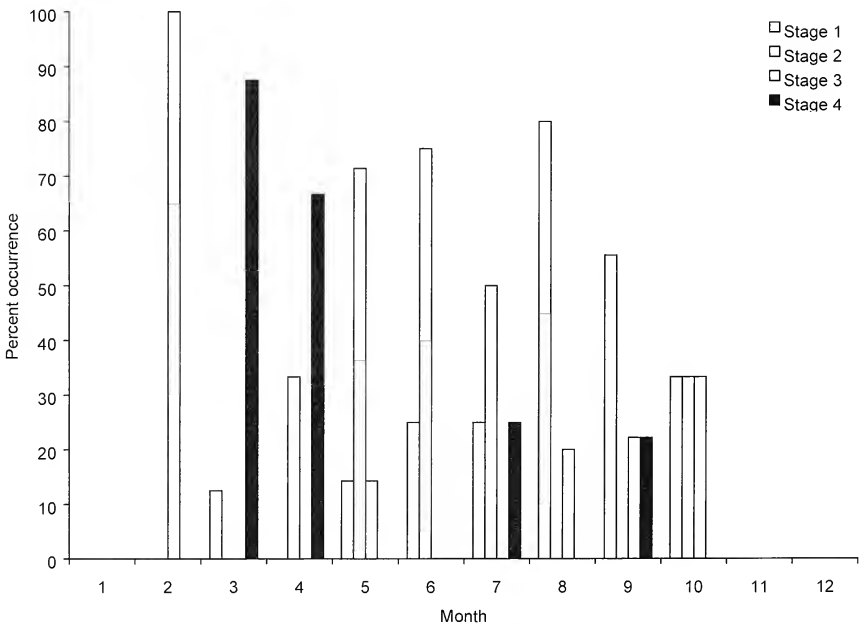


Figure 7. The relationship between mean ovum diameter and female body size in 10 female Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

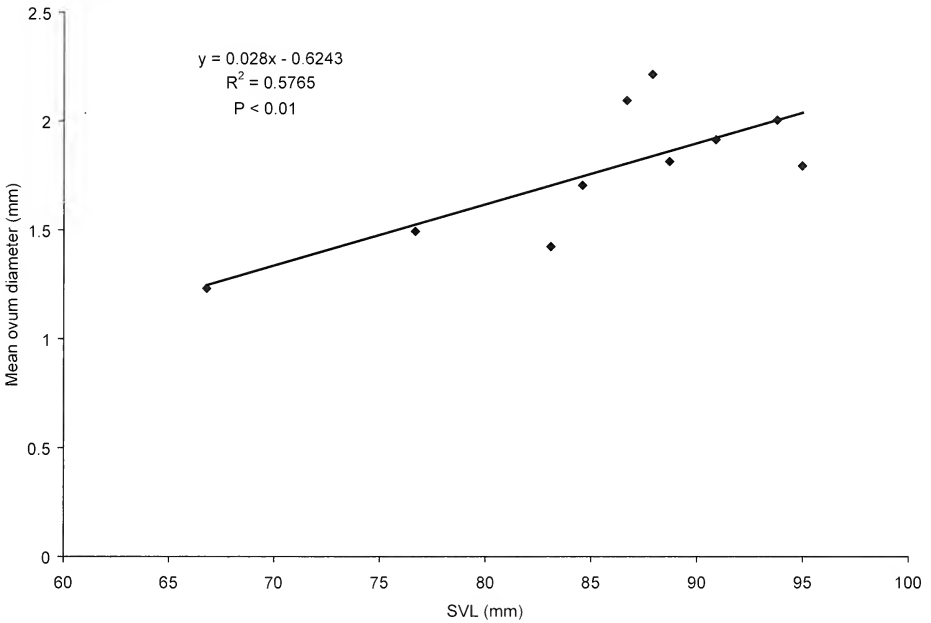
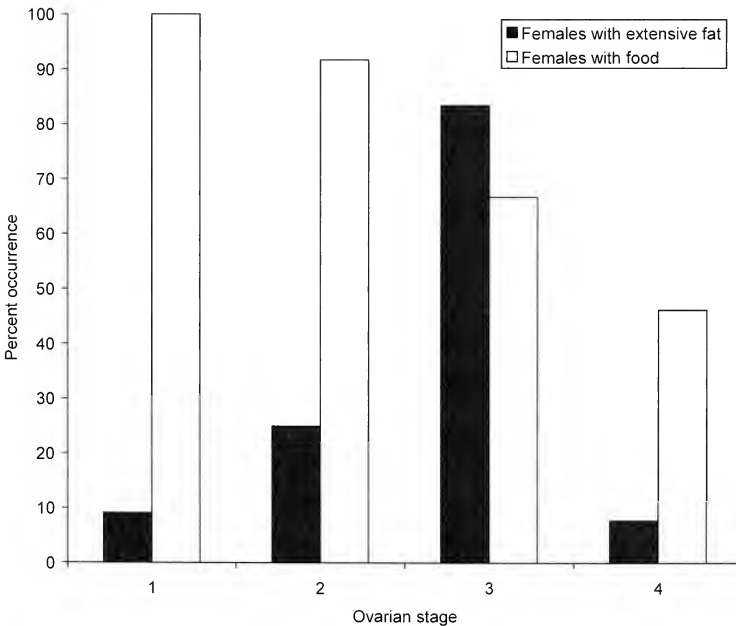


Figure 8. Frequency of extensive fat and the presence of food in each of the four ovarian stages of 42 female Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.

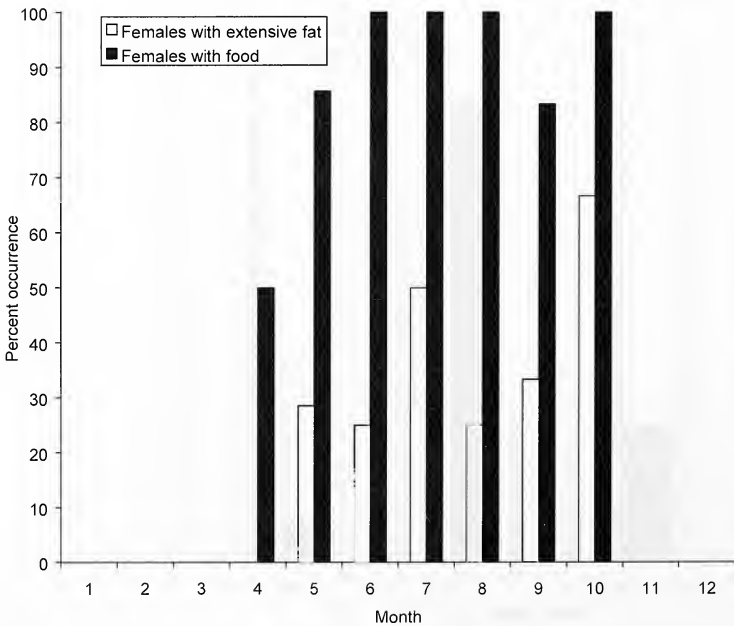


The monthly incidence of females containing extensive fat was highest just before hibernation (Figure 9). With the exception of a spike in this value in July, the pattern appeared to be one of depletion apparent in spring and early summer, which was followed by accumulation of fat from late summer onward (Figure 9). Except in early spring, monthly incidence of males containing prey was high throughout the active season (Figure 9).

Growth and sexual maturity.- Across Pennsylvania metamorphoslings were present in July and August (Figure 2, 10), indicating an approximately three month larval period after April and May breeding. Body sizes of metamorphoslings ranged 17.1-41.7 mm SVL (mean = 24.1 ± 3.9 mm SVL; n = 106). The monthly distribution of body sizes (Figure 10) suggests that males reached a minimum body size of 47.1 mm SVL within 12 months of larval transformation. Males reached their mean body size of 65.8 mm SVL (std. dev. = 7.9 mm SVL; range = 47.1-79.1; n = 54) at approximately 24 month of postmetamorphic age (Figure 10).

The smallest females that were yolking measured 49.0 (ovarian stage 2) and 53.0 mm SVL (ovarian stage 3). The smallest reproductively quiescent female (ovarian stage 1) measured 49.2 mm SVL, and the smallest gravid female (ovarian stage 4) measured 66.8 mm SVL. The monthly distribution of body sizes (Figure 10) east 49 mm SVL within one year of post-metamorphic age. However, all femalesizes (Figure 10) suggests that females could reach sexual maturity of at least 49 mm SVL within one year of post-metamorphic life. However, all females would be ready to breed in spring when 20-22 months of post-metamorphic age (Figure 10) at a body size near the smallest gravid female we examined and the mean adult body size of sexually mature females (mean = 71.5 + 14.1 mm SVL; range = 49.0-95.3; n = 47).

Figure 9. Monthly frequency of extensive fat and the presence of food in 39 female Northern Leopard Frogs (*Lithobates pipiens*) from Pennsylvania.



The mean body size, but not the variance (F-test, $P > 0.05$) of 15 gravid females (mean = 84.8 ± 8.6 mm SVL; range = 66.8-95.0) was significantly larger ($t = 1.679$, $df = 45$, $p < 0.001$) than that of non-gravid counterparts (mean = 65.3 ± 11.7 mm SVL; range = 49.0-87.8; $n = 32$). Among all sexually mature adults, significant differences were found in the variance ($F = 3.175$, $P < 0.001$) and mean ($t = -2.480$, $df = 70$, $p < 0.001$) of body size between in males and females.

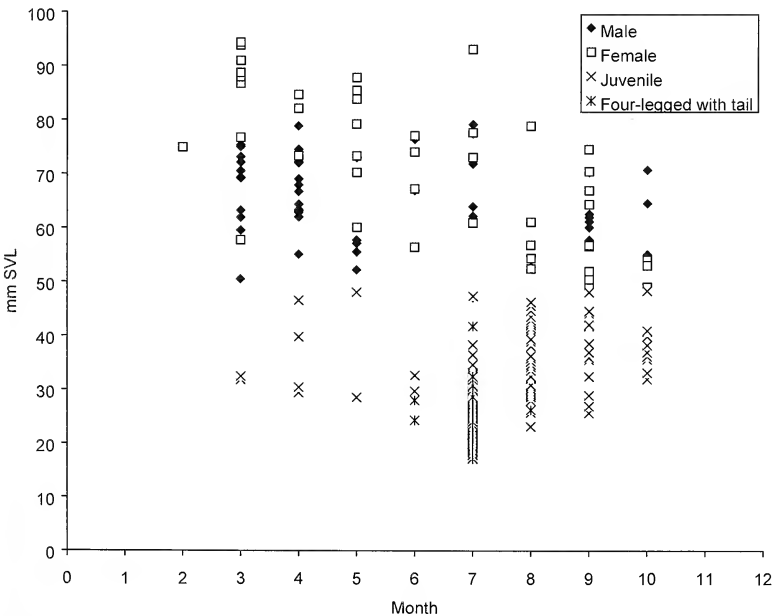
Discussion

The geographic range of the Northern Leopard Frog ranges southward from southern Quebec and extreme south of the District of Mack to New Mexico and Kentucky (Conant and Collins, 1998), thereby placing Pennsylvania near the southern edge of its geographic range.

Pennsylvania populations were active during February-October with collections of adults exhibiting a bimodal distribution in numbers. An active season of March-October has been reported for the species in both New England (Klemens, 1993) and Missouri (Johnson, 1987).

The March-April amplitude in collections in Pennsylvania overlapped the egg-laying season of this species. Females over-winter with eggs, and although gravid females were detected in July, the distribution of body sizes of juveniles, does not support breeding at that time. Evidence of spring breeding in Pennsylvania coincided with the generally short breeding season in the spring that typified breeding for the species in the United States, with a few notable exceptions in the Southwest (Rorabaugh, 2005). A summary of breeding dates corroborated a general pattern of spring breeding in the species but with a noticeable shift towards beginning and ending later in northern populations: March in Indiana (Minton, 2001) and Massachusetts (Klemens, 1993), March-April in Massachusetts

Figure 10. Monthly distribution of body sizes of males ($n = 54$), females ($n = 44$), juveniles ($n = 204$), and metamorphosings ($n = 106$) of the Northern Leopard Frog (*Lithobates pipiens*) from Pennsylvania.



(Dunn, 1930) and West Virginia (Green and Pauley, 1987), March-May in Illinois (Smith, 1961), April in Wisconsin (Vogt, 1981), April-June in Alberta, Canada (Russell and Bauer, 2000).

The Southern Leopard Frog, *Lithobates sphenoccephalus* (Cope, 1886), occurs in southeastern Pennsylvania, sympatrically with the Northern Leopard Frog in one county (Hulse et al., 2001; Meshaka and Collins, 2010). Like the Northern Leopard Frog, the southern form occurs over a large geographic range, where it replaces its northern congener in the American South (Conant and Collins, 1998; Butterfield et al., 2005). When we compared the breeding seasons of both species, we found that the breeding seasons of these two species were more similar to one another in Pennsylvania than they were to those of conspecifics at their respective geographic extremes. For the Southern Leopard Frog, breeding occurred primarily in April in Pennsylvania and during February-April in Delmarva (White and White, 2002): Generally spring up north and any time in the South, especially fall and again in spring (Butterfield et al., 2005).

Clutch size estimates for the Northern Leopard Frog have been reported to range 645-7645 eggs (Rorabaugh, 2005), and our estimates in range and mean fall within that range. In light of the large clutches and short breeding season in Pennsylvania, multiple clutch production seemed unlikely to us, which had it occurred, could have explained the absences of a significant relationship between clutch and female body size. More likely the small sample size could better explain the biologically meaningful even if not statistically significant positive trend in this relationship. On the other hand, ovum diameter increased with female body size, which we would have predicted would be reflected in the relationship between clutch and ovum size. Here again, a small sample size could explain the absence of significance in an otherwise weakly positive association. Ovum size in our sample, like clutch size, was similar to values provided from elsewhere in its geographic range: About 1.7 mm in Alberta (Russell and Bauer, 2000), 1.0-1.8 mm Wisconsin (Vogt, 1981), an average of 1.7 mm in Ohio (Walker, 1946).

Two to three months of larval growth was estimated for our sample. Our estimation for larval period for Pennsylvania Northern Leopard Frogs was similar to the estimates of 2-3 months in Indiana (Minton, 2001) and 70-100 days in Wisconsin (Vogt, 1981). Body size at transformation can vary widely in this species (Rorabaugh, 2005). Average body size of metamorphoslings in our sample was similar to the 25 mm average from a Quebec sample (Leclair and Castanet, 1987). However, the range in body sizes of metamorphoslings from our study ranged greater than that of the 25-32 mm range in Indiana (Minton, 2001) and ranged smaller than the 35-40 mm range that typified samples from Minnesota (Merrell, 1977). Transformation times in Pennsylvania were similar to the months of June-July in Wisconsin (Vogt, 1981), and June-August in Illinois (Smith, 1961).

In Pennsylvania, males and females matured at small body sizes and, once mature, mean body sizes of females were significantly larger than that of males. A comparison of body sizes among adults of males (mean = 63 mm; range = 51-87) and females (mean = 69.0 mm; range = 54-89) in Indiana (Minton, 2001) and males (mean = 57.2 mm; range = 51-65) and females (mean = 57.0 mm; range = 53-65) in Connecticut (Klemens, 1993) indicated that minimum body sizes among sites were variable within a small range, especially for males. However, unlike those of Connecticut (Klemens, 1993), Northern Leopard Frogs in both Indiana and our sample from Pennsylvania exhibited strong sexual dimorphism in body size with similarly strong male/ female body size ratio of 0.91 in Indiana (Minton, 2001) and 0.92 in our sample.

Like transformation size, age to sexual maturity in the Northern Leopard Frog varies widely across its geographic range. In Pennsylvania, the earliest hatching males and females were sexually mature in time to breed the following spring before reaching their first year of post-metamorphic life. Most individuals, however, reached sexual maturity after their first spring of post-metamorphic life, thereby missing breeding until their second spring just shortly before two years of post-metamorphic

life. For most populations, Northern Leopard Frogs reached sexual maturity at two to three years of age (Rorabaugh, 2005). Comparatively, in Ithaca, New York, maturity of a few individuals could be reached in the same year as metamorphosis (Ryan, 1953), and in Wisconsin males could reach sexual maturity in as early as one year after metamorphosis but generally at two years of age (Hine et al., 1981). Sexual maturity was attained at three years of age in northern Michigan (Force, 1933) and in two to three years after transformation in Alberta, Canada (Russell and Bauer, 2000).

In Pennsylvania, the Northern Leopard Frog is listed as a *Species of Greatest Conservation Need* (Morris, 2010), and a search through the literature has revealed very little work on any Pennsylvania populations. Consequently, this paper could represent a starting point in understanding the range of variability in the most basic of life history traits in Pennsylvania populations as well as a reference source for protocols to make detection for inventorying and monitoring this species more effective for researchers in Pennsylvania. This latter point is critical in light of regional and geographic variation evident in life history traits examined in this study.

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**Clutch characteristics of the Southern Leopard Frog,
Lithobates sphenoccephalus (Cope, 1886),
in Natchitoches, Louisiana**

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The Southern Leopard Frog, *Lithobates sphenoccephalus* (Cope, 1886) is an inhabitant of much of the United States, including Louisiana (Dundee and Rossman, 1989; Conant and Collins, 1991; Butterfield et al., 2005). In southern Louisiana, eggs are laid throughout the year with most reproductive activity occurring during December-February (Dundee and Rossman, 1989). In Louisiana, egg masses in the form of a plinth contain 1000-1500 eggs (Dundee and Rossman, 1989). Here, we provide clutch characteristics of six Southern Leopard Frogs from Natchitoches Parish, Louisiana. Specimens were derived from the vertebrate collection of Northwestern State University, Natchitoches, Louisiana, from females collected in February and April 1969 and February and June 1971. Female body size was measured in mm snout-vent length (SVL). Clutch size was estimated by weighing a subset of mature ova. Means are followed by ± 2 standard deviations.

Estimated clutch size of six gravid females (mean= 69.5 ± 7.70 mm SVL; range= 59.5-82.3) averaged 1585.4 ± 568.34 eggs (range= 975-2367.5). Clutch sizes in Natchitoches were smaller than those of northeastern Arkansas where clutch sizes of 39 females (51-89 mm SVL) averaged 2958.7 eggs (range = 1700-5537) (Trauth, 1989). However, too few gravid females were available from Natchitoches to make meaningful comparisons, and these findings underscore the opportunity that exists to conduct field and museum studies on this poorly studied but relatively common frog in Louisiana.

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The Effects of Temperature and Salinity on Wood Frog (*Lithobates sylvaticus*) Tadpole Growth and Survival

Abstract

Increased levels of road salt runoff in combination with increased temperatures earlier in the spring could create stressful environments for wood frog (*Lithobates sylvaticus*) tadpoles. We examined the effects of salinity and temperature as stressors, both independently and jointly. We used three concentrations of NaCl (control, low, and high) and two temperature treatments. Higher temperature resulted in significantly decreased survivorship, but did not affect mean tadpole mass. Salinity did not have any significant effects, nor did the interaction of salinity and temperature. These results suggest warming trends may have greater effects on this population than changes in salinity.

It is common for northern temperate regions to have salt-contaminated freshwater habitats due to the frequent use of road deicing compounds (e.g., Kaushal et al., 2005). Such salt-contamination can cause stunted growth, slower rates of metamorphosis, and decreased survival of anuran larvae (Dougherty and Smith, 2006; Collins and Russell, 2009; Langhans et al., 2009), however some species or populations of anurans are relatively tolerant of salt-contamination (e.g., Dougherty and Smith, 2006; Karraker, 2007; Collins and Russell, 2009). Indeed, some species appear to be prevented from occupying ponds contaminated with road salt, whereas other species appear to be able to occupy them (Collins and Russell, 2009).

Temperature is another factor that can affect the performance of anuran larvae. However, the effects of temperature on anuran tadpoles can be variable, with increased temperatures shown to decrease tadpole growth in some species (e.g., Álvarez and Nicieza, 2002; Orizaola and Laurila, 2009) and increase tadpole growth in other species (e.g., Sanuy et al., 2008; Castano et al., 2010). Temperature can also affect survivorship in tadpoles, with higher temperatures sometimes resulting in lower survivorship (e.g., Castano et al., 2010) or higher survivorship (e.g., Sanuy et al., 2008; Orizaola and Laurila, 2009).

Wood frogs (*Lithobates sylvaticus*) are a common and widespread frog in northeastern North America that inhabit vernal pools (Redmer and Trauth, 2005). This is a region where salinization of freshwater is important (Kaushal et al., 2005). Given that wood frogs are early spring breeders (Redmer and Trauth, 2005), they are likely to be affected by both road salt contamination and any warming trends associated with global climate change. Previous studies have found that wood frogs are susceptible to the effects of salt-contamination, but the concentrations that increase mortality or affect growth can vary among populations (e.g., Collins and Russell, 2009; Langhans et al., 2009; Petranka and Doyle, 2010). Road salt can also have significant demographic effects on wood frog populations (Karraker et al., 2008). Considering temperature, Castano et al. (2010) found that the survivorship of wood frog tadpoles from Ohio was better at 17°C than at 25°C; however, they found that tadpoles at 25°C were larger than tadpoles at 17°C.

To our knowledge, no previous study has examined the potential interaction between salt contamination and temperature on the performance of wood frog tadpoles. Such information may allow us to better understand the potential impacts of these environmental stressors on wood frogs.

To this end, we examined how the combination of increased salinity and increased water temperature may affect the growth and survivorship of wood frog tadpoles.

Materials and Methods

We collected wood frog egg masses ($N = 6$) from a local pond within 24 h of oviposition. We incubated the eggs in the laboratory until hatching. Upon hatching, tadpoles were maintained in large plastic containers where tadpoles from the different clutches were allowed to mix. We began the experiment once tadpoles reached Gosner Stage 26 (Gosner, 1960) and had a mean mass of 0.023 ± 0.001 g ($N = 10$).

The experiment was a 3×2 fully factorial design with three salinity treatments (control, low, and high) and two temperature treatments (25°C and 30.5°C) replicated 6 times. For the salinity treatments, we created stock solutions of the low (500 mg NaCl/L) and high (1000 mg NaCl/L) salinity treatments using NaCl (Fisher Scientific Sodium Chloride Certified for Biological Work) and aged tapwater. Temperature treatments were created by placing the containers in either a thermostat controlled lab (25°C) or a thermostat controlled greenhouse (30.5°C). Each experimental unit consisted of a clear plastic container (21 cm x 14 cm x 5 cm) filled with 500 mL of the appropriate salinity solution. Each container had 5 tadpoles.

Every 3 days we refilled each container with water of the appropriate salt concentration that had been acclimated to the treatment temperature. We also removed feces and any remaining food. We fed the tadpoles 0.05g of crushed Purina Rabbit Chow per tadpole every 3 days. After 10 days, we recorded the number of tadpoles alive in each container and weighed the survivors to the nearest 0.001 g after blotting dry. We used two-way ANOVAs to analyze the effects of temperature and salinity on tadpole mass and survivorship separately.

Results

Survivorship to the end of the experiment was higher in the 25°C treatments than in the 30.5°C treatments (25°C : 0.80 ± 0.063 [$N = 18$], 30.5°C : 0.267 ± 0.096 [$N = 18$]; $F_{1,30} = 22.26$, $P < 0.0001$). Salinity had no effect on tadpole survivorship (Control: 0.65 ± 0.14 [$N = 12$], Low: 0.47 ± 0.12 [$N = 12$], High: 0.48 ± 0.11 [$N = 12$]; $F_{2,30} = 1.49$, $P = 0.24$). The interaction between temperature and salinity was not significant ($F_{2,30} = 1.18$, $P = 0.32$).

Mean tadpole mass was not significantly affected by temperature treatment (25°C : 0.0530 ± 0.0037 g [$N = 17$], 30.5°C : 0.0614 ± 0.0085 g [$N = 6$]; $F_{1,17} = 1.05$, $P = 0.32$). Mean tadpole mass was also not affected by salinity treatment (Control: 0.052 ± 0.0009 g [$N = 8$], Low: 0.053 ± 0.006 g [$N = 7$], High: 0.060 ± 0.009 [$N = 8$]; $F_{2,17} = 0.98$, $P = 0.40$). The interaction term was not significant ($F_{2,17} = 0.50$, $P = 0.61$).

Discussion

Our results indicate that NaCl concentrations of 500 and 1000 mg L^{-1} did not affect the growth or survivorship of wood frog tadpoles. This is in contrast to other studies that have found salinity to negatively affect survivorship, growth, and size at metamorphosis in wood frogs at concentrations ranging up to 1400 mg L^{-1} (Sanzo and Hecnar, 2006; Karraker et al., 2008). However, Petranka and Doyle (2010) found increased mortality in wood frog tadpoles only at concentrations of 4500 mg L^{-1} and no effect at lower concentrations. Thus, there appears to be a range of susceptibility to salinity in wood frog tadpoles across their geographical range. What drives such variability in susceptibility is unknown but warrants further investigation.

The survivorship of wood frog tadpoles at 25°C was greater than their survivorship at 30.5°C, but mean tadpole mass did not differ between the temperatures. Castano et al. (2010) found that survivorship of wood frog tadpoles was better at 17°C than at 25°C; however, they found that tadpoles at 25°C were larger than tadpoles at 17°C. Our results for survivorship are generally consistent with Castano et al. (2010) in that survivorship is better at the cooler temperature. Our results are also consistent with the observation that 25°C is near the maximum tolerated temperature for wood frog tadpoles from Ohio (Manis and Claussen, 1985). Thus, higher temperatures appear to negatively affect survivorship in wood frog tadpoles. However, our results for mass and those from Castano et al. (2010) suggest that growth in wood frog tadpoles in this Ohio population is generally better at higher temperatures since growth at 25°C was greater than at 17°C (Castano et al., 2010) and similar to that at 30.5°C (this study). The results of these two experiments do suggest that increasing temperatures associated with a warming climate could have serious consequences for wood frogs, especially since the increases in growth performance appear to level off after 25°C. However, spring temperatures for this Ohio population would have to drastically increase to have substantial impacts on wood frogs (water temperatures for the source pond in this experiment averaged 13.7°C in early April; Dougherty et al., 2005).

In summary, we found that wood frogs are not strongly affected by NaCl concentrations of 500 and 1000 mg/l, but they do suffer a reduction in survivorship when exposed to a warmer temperature of 30.5°C in comparison to 25°C. There does not however appear to be a synergistic effect of these stressors on wood frogs in this population, as evidenced by the lack of a significant interaction between temperature and salinity.

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Body temperatures of *Hyla arenicolor* from Sierra de Tepozotlan, Estado de Mexico, Mexico

Body temperature affects numerous aspects of locomotory and physiological performance of *Hyla* treefrogs (Navas 1996b). In addition, climatic conditions, including temperature, are likely to drive the differential distributions of some *Hyla* species (Otto et al. 2007). It is thus important to gain a better understanding of the temperature relationships of *Hyla* frogs. However, there is relatively little known about temperature relationships in the genus *Hyla*. Here we report on the temperature relationships of *Hyla arenicolor* from Sierra de Tepozotlan, Estado de México, Mexico in an effort to expand the database of temperature relationships in treefrogs.

Materials and Methods

We conducted this study in Sierra de Tepozotlán (19° 42' 23.4" N, 99° 15' 17.6" W and 2300 m elevation), in Estado de México, Mexico. Mean annual temperature and precipitation are 16°C and 650 mm, respectively. Plant species include *Quercus crassipes*, *Q. microphylla*, *Q. rugosa*, *Bouteloua curtipendula*, *B. gracilis*, *B. hirtusa*, *Lycurus phleoides*, *Piptochaetium fimbriatum*, *Aegopogon cenchrroides*, *Festuca* sp., *Piptochaetium fimbriatum*, *Bromus* sp., *Aristida* sp., *Oennetum clandestinum*, *Eragrostis* sp. and *Hilaria cenchrroides*, principally (Rzedowski 2006).

We captured frogs by hand. Once captured, we recorded snout vent length (SVL, to nearest 1 mm), body mass (to nearest 0.2 g, using a spring balance), and body (T_b ; cloacal temperature, to nearest 0.2°C), air (T_a ; bulb in the shade, 3.0 cm over the substrate occupied by the lizard, to nearest 0.2°C), and substrate temperature (T_s ; bulb to the shade on the substratum occupied by the small lizard, to nearest 0.2°C) using a quick-reading thermometer (Shultetheis, Miller and Weber Inc., interval 0-50°C, 0.2 precision). We also recorded each frog's position with regard to solar insolation as being completely exposed to sun, in shade, or in a sun/shade mosaic. Frogs that needed a major effort to capture (> 1 min.) were excluded from temperature records. We used only one observation for each frog.

Results and Discussion

Mean T_b was 25.11 ± 0.34 °C (N = 48). Mean T_a was 19.04 ± 0.32 °C (N = 48). Mean T_s was 21.69 ± 0.35 °C (N = 48). Mean T_b for our population of *H. arenicolor* is higher than that observed in a population of *H. arenicolor* from Colorado (20.7°C; Snyder and Hammerson 1993). Our observed mean T_b is within the range of T_b s observed in *Hyla microcephala* and *H. ebraccata* from low elevations in Panama (Navas, 1996b), but is higher than in the high elevation *H. lavialis* (Valdivieso and Tamsitt 1974; Navas 1996a,b). *Hyla regilla* from southern California had T_b s that ranged from 14.3 – 22.2 °C (Brattstrom and Warren 1955). *Hyla cinerea* from Louisiana had nocturnal T_b s ranging from 19.1 – 27.7°C (Wygoda and Williams 1991).

Body temperature increased with T_a (N = 48, $r^2 = 0.18$, $P = 0.0022$; $T_b = 16.26 + 0.46T_a$). Body temperature also increased with T_s (N = 48, $r^2 = 0.28$, $P = 0.0001$; $T_b = 14.02 + 0.51T_s$). The T_b s of *H. arenicolor* from Colorado tended to be higher than T_a but lower than T_s (Snyder and Hammerson 1993). The dependence of T_b on T_a has been found in other *Hyla* species (Valdivieso and Tamsitt 1974; Wygoda and Williams 1991; Navas 1996a).

Body temperature increased with frog SVL, but only a small amount of variation in T_b was explained by SVL (N = 48, $r^2 = 0.08$, $P = 0.046$; $T_b = 22.01 + 0.13SVL$). Body temperature was not related to frog mass (N = 46, $r^2 = 0.04$, $P = 0.16$). Body temperature was not affected by

SVL in *H. labialis* (Valdivieso and Tamsitt 1974).

Most frogs were observed in sunny microhabitats (30; 62.5%), followed by mosaic microhabitats (12; 25%). Few frogs were observed in shade (6; 12.5%). Microhabitat did not affect T_b (Table 1; $F_{2,45} = 1.86$, $P = 0.17$), T_a (Table 1; $F_{2,45} = 0.06$, $P = 0.94$), or T_s (Table 1; $F_{2,45} = 0.99$, $P = 0.38$). Snyder and Hammerson (1993) found all of the *H. arenicolor* they observed in June in Colorado in full sun.

In conclusion, the temperature relationships we observed in *H. arenicolor* from Tepozotlan, Estado de México, Mexico, are fairly similar to previous studies on the thermal ecology of *H. arenicolor* and other species of *Hyla*.

Table 1. Mean body temperature (T_b), air temperature (T_a), and substrate temperature (T_s) of *Hyla arenicolor* found in sunny, sun/shade mosaic, and shaded microhabitats. Means are given ± 1 SE.

	T_b (°C)	T_a (°C)	T_s (°C)
Sunny (N=30)	25.41 \pm 0.35 °C	18.96 \pm 0.41 °C	22.06 \pm 0.38 °C
Sun/Shade Mosaic (N=12)	25.22 \pm 0.83 °C	19.16 \pm 0.63 °C	21.24 \pm 0.83 °C
Shaded (N=6)	23.42 \pm 1.19 °C	19.25 \pm 0.93 °C	20.07 \pm 1.27 °C

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Clutch characteristics of the Pickerel Frog, *Lithobates palustris* (LeConte, 1825), in Natchitoches, Louisiana

The Pickerel Frog, *Lithobates palustris* (LeConte, 1825) is an inhabitant of eastern North America, including Louisiana (Dundee and Rossman, 1989; Conant and Collins, 1991; Redmer, 2005). In northern Louisiana, breeding was reported during February-April and thought to occur in the winter in southerly regions of the state (Dundee and Rossman, 1989). In Caddo Parish of northern Louisiana, reproductive movements by females occurred during December-April (Hardy and Raymond, 1991). In Louisiana, eggs are laid in firm globular masses of 2,000-3,000 eggs (Dundee and Rossman, 1989). Here, we provide clutch characteristics of two Pickerel Frogs from Natchitoches Parish, Louisiana. Two specimens were derived from the vertebrate collection of Northwestern State University, Natchitoches, Louisiana. Female body size was measured in mm snout-vent length (SVL). Clutch size was estimated by weighing a subset of mature ova. Means are followed by ± 2 standard deviations.

The larger female (58.7 mm SVL), captured on 19 March 1969, contained an estimated 1166.7 eggs. The smaller female (53.1 mm SVL), captured on 6 April 1971, contained and estimated 875 eggs.

Our data from Natchitoches did not conflict with the general range of adult females or generally small clutches found in Arkansas (range= 960-2943 eggs) (Trauth et al., 1990), Missouri (range= 704-2896 eggs) (Resetarits and Aldridge, 1988), and Pennsylvania (range= 850-2450 eggs) (Meshaka et al., 2010). Our findings do not conflict with an apparent stability in clutch characteristics in this species (Meshaka et al., 2010).

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Distribution of Tadpoles (*Hyla arenicolor*) in the Pools Associated with the Río Salado, Puebla, Mexico

One of the goals of studying the ecology of anurans is to understand why it's distributed in some sites and not in others. It has been documented that factors such as pH, temperature, photoperiod, dissolved oxygen, and salinity, among others, are involved in the distribution of tadpoles (Welch and MacMahon, 2005; Girish and Krishnamurthy, 2009; Woolrich-Piña et al., 2010).

The Río Salado is characterized by a high level of salinity present in the low basin (1.0-6.0 ppt) due to the erosion of carbonated sediment from the medium and high basin as well as for the production of salt (Woolrich-Piña, 2010). This might influence the distribution of the anurans that inhabit the different pools associated with the river. *Hyla arenicolor* is one of four species of anurans that are distributed along the Río Salado, in the Valle de Zapotitlán Salinas, Puebla, México. Here we report which are the factors that influence the distribution of *H. arenicolor* (tadpoles) in the Río Salado.

Materials and Methods.

The study was realized in San Juan Raya, municipality of Zapotitlán Salinas Valley (18° 18' N, 97° 37' W and 1730 m elevation), in Puebla, Mexico. Mean annual temperature and precipitation are 21°C and 400 mm, respectively. Plant species include some cacti (*Nebouxbania tetetzo*, *Cephalocereus* spp.), mesquite trees (*Prosopis laevigata*), and pata de elefante trees (*Beucarnea gracilis*), principally (Rzedowski 2006).

We conducted surveys along a 1 km segment of the Río Salado monthly from March to June 2010 to characterize conditions in the river and determine the distribution of tadpoles of *H. arenicolor*. The conditions characterized were: length, width and depth (cm), salinity (ppt), and dissolved oxygen (mg/L⁻¹) of each pond. Salinity, and dissolved oxygen were measured using a YSI Model 85 Handheld DO/ conductivity meter.

We used a multivariate analysis of variance (MANOVA) to compare the physical and chemical parameters between pools with and without tadpoles among months. A significant MANOVA was followed by unifactorial nested ANOVAS to examine each variable in detail.

Results.

MANOVA found significant differences in the pools with and without tadpoles (Wilks' $\lambda = 0.008$, $F_{28,132} = 25.13$, $P < 0.01$). Tadpoles were found in pools deeper ($F_{1,52} = 23.8$, $P < 0.01$), wider ($F_{1,52} = 5.73$, $P < 0.01$), longer ($F_{1,52} = 5.68$, $P < 0.01$), with higher dissolved oxygen (DO) levels ($F_{1,52} = 40.3$, $P < 0.01$) and lower salinity ($F_{1,52} = 348.7$, $P < 0.01$) than pools without tadpoles (see table 1).

Discussion.

Hylid tadpoles were observed in pools with low salinity. This has been observed in other anuran species, because it generally does not frequent environments with high salt concentrations (Davenport and Huat, 1997; Smith et al., 2007; Wells 2007). However, it has been observed that salinity did not affect the distribution of *Buergeria japonica* tadpoles, the abundance of *Rhinella marina* increased with salinity, and *Fejervarya cancrivora* tolerates it in very high concentrations (Gordon et al., 1961; Haramura, 2007; Ríos-López, 2008).

We also found tadpoles in pools with higher DO levels. This is consistent with other species (e.g. *Incilius [Ollotis] occidentalis*, *Rana pipiens* and *Anaxyrus terrestris*; Noland and Ultsch, 1981; Woolrich-Piña et al., 2010). Dissolved oxygen can be related to species richness and predation on tadpoles (Ultsch et al., 1999; Peltzer and Lajmanovich, 2004).

Tadpoles were distributed in longer, wider and deeper pools. A pool with a greater volume has a lower probability of dessication, increasing the time for larval development. Premature pool drying is often a major cause of mortality for the larvae of many species (Beebee, 1996).

Due to the importance of water chemistry in the distribution of *H. arenicolor* tadpoles, it is important to consider the potential effect of salt factories on water quality in the river. Salt factories ("salineras") divert water from the Río Salado to harvest the salt by evaporation. Thus it is possible that the salineras may impact the habitat of native fauna, including *H. arenicolor*.

Table 1. Mean values and interval of the chemicals and physicals parameters present in the ponds.

Pond	O mg/L-1	Salinity ppt	Length (m)	Width (m)	Depth (m)
With tadpoles	4.7 ± 0.4	(1.2 – 2.8)	4.1 ± 0.7	(0.8 – 5.9)	6.4 ± 1.6
	(1.3 – 15)	1.1 ± 0.6	(0.43 – 3.2)	0.26 ± 0.05	(0.25 – 1)
Without tadpoles	2.3 ± 0.7	(3.3 – 6.5)	0.9 ± 0.08	(0.2 – 0.9)	3.2 ± 0.9
	(0.8 – 4.7)	2.5 ± 0.6	(0.84 – 6.4)	0.55 ± 0.08	(0.08 – 0.42)

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Mississippi Map Turtle, *Graptemys pseudogeographica kohnii*, Documented in Frederick County Maryland

A non-native species of turtle was observed and photographed in the Monocacy River at Links Bridge Road Frederick County Maryland (lat. 39.534046° lon. -77.353774°) on 29 Aug 2010 while conducting field research for the Maryland Amphibian and Reptile Atlas (MARA). The low resolution photograph showed the specimen to be one of the Map Turtles. To confirm species identification I returned to the same location the following day with a telephoto camera. I observed and photographed two *Graptemys pseudogeographica kohnii*, note the characteristic crescent shape mark behind the eye (Figures 1 and 2). Further research is needed to determine if this report represent a viable colony.

The indigenous range for the Mississippi Map Turtle, a sub species of the False Map Turtle, is the Mississippi River basin from western Tennessee into Missouri and Nebraska and south to the Gulf of Mexico in Texas, Louisiana, and Mississippi. *Graptemys pseudogeographica kohnii* were previously documented in Calvert County Maryland (Schwartz and Dutcher, 1960) and near Washington D.C. in the Middle Potomac-Anacostia-Occoquan area (Mitchell, 1994), however this report represent the first for Frederick County Maryland.

Figure 1. Mississippi Map Turtle, *Graptemys pseudogeographica kohnii*



Figure 2. Mississippi Map Turtle, *Graptemys pseudogeographica kohnii*.



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Reproduction in Clark's Spiny Lizard, *Sceloporus clarkii* (Squamata: Phrynosomatidae) From Sinaloa, Mexico

Abstract.

A histological examination of gonads from Clark's spiny lizard, *Sceloporus clarkii*, from Sinaloa, Mexico revealed an extended period of spermiogenesis (sperm formation) that extended from spring into late summer. Recrudescence (renewal of germinal epithelium in the seminiferous tubules for the next period of spermiogenesis) began in winter. Yolk deposition (enlarged follicles > 5 mm) was underway in June. Ovarian activity continued into August. Histological evidence indicates that *S. clarkii* produces multiple clutches in the same year. The mean clutch size for 18 gravid females was 9.56 ± 2.60 , range = 7-18. Females of *S. clarkii* from Sinaloa mature at a smaller size, produce smaller clutches and produce clutches later in the year than do Arizona populations.

Introduction.

Clark's spiny lizard, *Sceloporus clarkii* Baird and Girard, 1852 ranges from central Arizona and southwest New Mexico, south to Jalisco, Mexico from sea level to ca. 1830 m; (Stebbins, 2003). It tends to prefer oak-pine woodlands, tropical deciduous forests and subtropical thorn forests of lower mountain slopes (Stebbins, 2003). Anecdotal accounts of *S. clarkii* reproduction appeared in Kauffeld (1943), Stebbins (1954, 2003), Fitch (1970, 1985), Hulse (1973), Parker (1973), Vitt (1977), Behler and King (1979), Degenhardt et al. (1996), Brennan and Holycross (2006), Schwable and Rosen (2009). The most detailed study on the reproduction of *S. clarkii* was by Tinkle and Dunham (1986) on a population from central Arizona. Fitch (1985) reported larger clutch sizes from northern (Arizona) versus southern (Mexico) populations of *S. clarkii*. The purpose of my study is to compare aspects of the reproductive biology of *S. clarkii* from central Arizona (Tinkle and Dunham, 1986) with that of a conspecific population in Sinaloa, Mexico approximately 1200 km southward. Information on timing of sperm production, clutch size and body (SVL), size at maturity provides life history data that may be useful in elucidating phylogenetic affinities.

Methods.

A sample of 79 adult *S. clarkii*, consisting of 43 males (mean SVL = 93.4 mm \pm 12.7 SD, range = 63-124 mm) and 36 females (mean SVL = 84.8 mm \pm 9.9 mm, range = 71-111 mm) and an additional six sub-adults (mean SVL = 63.0 mm \pm 6.5 SD, range = 52-68 mm), all collected during the period 1933-1979 from Sinaloa, Mexico was examined (Appendix).

For histological examination, the left testis was removed from males and the left ovary was removed from females. Enlarged follicles (> 5 mm length) or oviductal eggs were counted (*in situ*). Tissues were embedded in paraffin and cut into sections of 5 μ m.

Slides were stained with Harris' hematoxylin followed by eosin counterstain (Presnell and Schreibman, 1997). The slides of testes were examined to determine the stage of the spermatogenic cycle while the slides of ovaries were examined for the presence of yolk deposition or corpora lutea. Histology slides were deposited in LACM. An unpaired *t*-test was used to compare *S. clarkii* male and female body sizes (SVL)s. The relationship between female body size (SVL) and clutch size was investigated by linear regression analysis. Statistical tests were performed using InStat (vers. 3.0b, Graphpad Software, San Diego, CA).

Results.

The *S. clarkii* males were significantly larger than the females (unpaired *t*-test, $df = 78$, $t = 3.31$, $P = 0.001$). Monthly stages in the testicular cycle are in Table 1. Three stages were observed in the testicular cycle: (1) regressed in which the seminiferous tubules contain mainly spermatogonia interspersed with Sertoli cells; (2) recrudescence in which proliferation of germ cells for the next period of sperm formation has commenced. Primary spermatocytes and occasional secondary spermatocytes predominate. In late recrudescence (April) some spermatids are noted; (3) spermiogenesis in which the lumina of the seminiferous tubules are lined by clusters of sperm or clusters of metamorphosing spermatids. Recrudescence was noted in winter and early spring (Table 1). Sperm formation (spermiogenesis) began in May and continued into August. Regressed testes appeared in August, September and December (Table 1). The smallest reproductively active male measured 63 mm SVL (LACM 6623) and was from June. One slightly smaller male, 58 mm SVL (LACM 8631) from April with a regressed testis was considered a subadult.

Monthly stages in the ovarian cycle of *S. clarkii* are listed in Table 2. Five stages were observed: (1) quiescent, in which there is no yolk deposition; (2) early yolk deposition, in which vitellogenic granules are accumulating within the follicles; (3) enlarged follicles > 5 mm; (4) oviductal eggs; and (5) corpora lutea (previous clutch) and concomitant yolk deposition for a subsequent clutch. Reproductively active females were present in June, July and August. Because spring samples were lacking, it was not possible to determine when females commenced reproductive activity. One female from 2 August (LACM 6633) contained corpora lutea from a previous clutch and concomitant yolk deposition for a subsequent clutch while a second female (LACM 6634) from 3 August contained oviductal eggs with concomitant yolk deposition for a subsequent clutch both indicating that *S. clarkii* from Sinaloa can produce multiple clutches in the same reproductive season (Table 2). The mean clutch size for the 18 gravid females was 9.56 ± 2.60 , range = 7-18. The relationship between female SVL and clutch size was not significant ($n = 18$, $P = 0.0744$). The smallest reproductively active female *S. clarkii* (LACM 6647) collected in August contained 8 enlarged follicles (> 5 mm) and measured 73 mm. Three smaller females with quiescent ovaries collected in April (SVL = 66-68 mm) were considered to be subadults.

Discussion.

Males of *S. clarkii* are similar to other North American lizards that exhibit spring-summer spermiogenesis (Goldberg 1974, 1975, 1976, 1977, 1983). However, they differ in the extended length of sperm production, in Sinaloa, which continues into August.

Table 1. Monthly stages in the testicular cycle of 43 *Sceloporus clarkii* from Sinaloa, Mexico.

Month	N	Regressed	Recrudescent	Spermiogenesis
January	1	0	1	0
March	1	0	1	0
April	3	0	3	0
May	1	0	0	1
June	8	0	0	8
July	13	0	0	13
August	14	5	0	9
September	1	1	0	0
December	1	1	0	0

Fitch (1970) reported one of four *S. clarkii* females collected in November from Mexico (locality not specified) in a series from Chihuahua, Durango and Sinaloa as gravid. Sixty-seven percent ($n = 15$) of August females from Sinaloa were reproductively active (Table 2). *Sceloporus clarkii* females in Sinaloa mature at 73 mm SVL as was also reported by Fitch (1970). In contrast, Tinkle and Dunham (1986), reported that females from central Arizona, finished reproducing in late July; one female had oviductal eggs on 1 August. In *S. clarkii* from central Arizona females mature at 90 mm SVL (Tinkle and Dunham 1986), a larger size than in Sinaloa. Clutch sizes of *S. clarkii* from Mexico were smaller than those produced by females in Arizona (Table 3).

In conclusion, my data indicate variation in the reproductive cycles of *S. clarkii* from central Arizona (Tinkle and Dunham 1986) versus Sinaloa, Mexico. Females from Sinaloa mature at a smaller size, produce smaller clutches and produce clutches later in the year than do Arizona populations.

Table 2. Monthly stages in the ovarian cycle of 36 *Sceloporus clarkii* from Sinaloa, Mexico.

Month	<i>n</i>	Quiescent	Early yolk deposition	Enlarged follicles > 5mm	Oviductal eggs	Corpora lutea and yolk deposition
June	6	3	0	1*	2	0
July	12	0	4	2	6**	0
August	15	5	0	5	4***	1
September	2	2	0	0	0	0
October	1	1	0	0	0	0

*Follicles were damaged and could not be counted; **One group of oviductal eggs were damaged and could not be counted; ***One female contained oviductal eggs and concomitant yolk deposition for a subsequent clutch.

Table 3. Mean clutch sizes for *Sceloporus clarkii* from different parts of its range.

Location	<i>n</i>	Mean Clutch Size	Range	Source
Mexico (Chihuahua, Durango, Sinaloa)	6	8.0	1-10	Fitch, 1985
Sinaloa	18	9.6	7-18	This paper
Central Arizona	32	19.6	7-28	Tinkle and Dunham, 1986
Southern Arizona	17	15.2	8-24	Fitch, 1985

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Appendix

Sceloporus clarkii from Sinaloa, Mexico examined from the Natural History Museum of Los Angeles County (LACM), Los Angeles, California: 6620-6626, 6628-6641, 6644, 6645, 6647-6657, 7303, 8626, 8628, 8631-8636, 17396, 17397, 17400, 37618, 50992, 51012-51014, 65182, 74296, 92952, 95628-95635, 95637-95650, 95652-95655, 95953, 121323, 133272-133274.

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Harassment/Predation of Maryland Snakes by Bird Species

Elsewhere, there are many accounts of predation on snakes by birds. Eagles, Falcons, Hawks....all birds of prey are well known snake eaters. Ravens, Crows, Magpies (all the corvid types) and Kookies all eat small snakes. Egrets, Herons and Storks are also known snake eaters. A quick check online, indicated that Wikipedia, the Free Encyclopedia (2011) listed Red Tailed Hawks, Red Shouldered Hawks, Secretary Birds, Shrikes, Stellar's Sea Eagle, Bald Eagle, Osprey, Blue Heron, American Egret, Cattle Egret, Green Heron, Sandhill Crane, Limpkin and Wood Stork as snake eaters.

Shine et al.(2000, 2001) noted that in Manitoba, during the spring mating season, intense predation primarily by crows (*Corvus brachyrhynchos*) on mainly small snakes. They stated that "Crows generally removed the snake's liver and left the carcass....".

Seeing birds preying on DOR snakes is quite common, while observing the attacks on living snakes is probably relatively more common than observations would indicate.

On 12 April 2005 I observed an Eastern Crow (*Corvus brachyrhynchos brachyrhynchos*) feeding on the carcass of a DOR *Scotophis alleghaniensis* on Dicus Mill Road, 0.2 mi South Pyles Lane, Anne Arundel County, Maryland, something seen very commonly along all roads.

Not as common, is the predation on snakes by birds. On 17 September 2004 I observed a group of five crows (*Corvus brachyrhynchos brachyrhynchos*) deliberately attacking a six foot *Scotophis alleghaniensis* on Grover Road at Brightview Drive, Anne Arundel County, Maryland.. This snake was thin and appeared debilitated. It however kept backing up and finally was able to disappear under the vegetation along the side of the road. The crows kept grabbing the tail trying to pull in back on the road, but were unsuccessful.

A US Postal Service employee, John Dirks relayed an account from 1:30 PM on 17 May 2011. On walking up the walk way to a house at 136 Drexel Drive, Millersville, Anne Arundel County, Maryland, he encountered two Eastern Crows (*Corvus brachyrhynchos brachyrhynchos*) harassing a 4 foot *Scotophis alleghaniensis*. The crows seemed persistent and were still pursuing the snake when he left. He said the snake seemed to be in good health.

Another US Postal Service employee, Carl Carlson, relayed two observations made on consecutive days. He has tentatively identified the hawks involved as Red-Shouldered Hawks (*Buteo lineatus*) and the black snakes as *Scotophis alleghaniensis*. Carl is a cigar smoker, and pauses to enjoy both nature and his cigar! On 18 March 2010 at 12:00 Noon, he observed a large hawk, carrying a black snake, fly to it's nest at the top of a large tree off Zeman Road, just off Obrecht Road, Anne Arundel County, Maryland. On 19 March 2010, at 12:30 PM, he observed a large hawk, carrying a black snake, fly up on to the roof of a house at 6 Forham Court, off West Pasadena Road, Anne Arundel County, Maryland, where it proceeded to devour the snake. These snakes were in the 4 foot size range. This is perhaps a danger faced by many snakes emerging from hibernation enjoying the rays of the sun out in the open, and during the spring matting season as mentioned above (Shine, (2000, 2001).

And now to the most interesting account concerning a Cat Bird (*Dumetella carolinensis*). Boris Stegmar told me of an observation he made on 17 August 2011. The observation was made in the early afternoon at 1152 River Bay Road, Annapolis, Maryland. He watched as a Cat Bird repeatedly harassed a three foot *Scotophis alleghaniensis*. The snake kept retreating while

the Cat Bird followed in pursuit pecking the snake's tail. This went on for about five minutes until the snake managed to escape. I would imagine that Cat Birds are normal prey items for *Scotophis alleghaniensis* so this appears to be abnormal behavior, or perhaps it had a nest that was threaten by the snake.

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News and Notes:

Call for Papers

This is the first time in forty six years, as Editor of the Bulletin of the Maryland Herpetological Society, that I did not receive enough material to put out four Numbers to this Volume. This is a plea to all of you for help in the coming year. The Bulletin has been a part of the NHSM's Department of Herpetology and we really would like to see it continue. Thank you for your past support and please answer this call for additional support. Thank you. The Editor.

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