

Growth, reproduction, and life span in Blanchard's Cricket Frog (*Acris blanchardi*) with notes on the growth of the Northern Cricket Frog (*Acris crepitans*)

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Abstract. Blanchard's Cricket Frog (*Acris blanchardi*) and the Northern Cricket Frog (*Acris crepitans*) are small frogs commonly found along water bodies in eastern North America. We determined growth and seasonal size classes from museum specimens of the Northern Cricket Frog (from Georgia and Florida) and Blanchard's Cricket Frog (from Arkansas and Missouri). We characterized the male and female reproductive phenophases of Blanchard's Cricket Frog using histological technique and gross examination and assessed its age and growth using skeletochronology. Our results show that male and female Blanchard's Cricket Frogs metamorphose in the summer, and snout-vent (SVL) length quickly reaches adult size. Body mass (BM) follows SVL with female BM growing faster than males from spring through oviposition. Male and female reproductive phenophases follow growth patterns. Some males are reproductively viable by late summer. Most ovarian development occurs in the spring and summer with oviposition occurring sometime between late May and June. Growth, reproductive and skeletochronological evidence suggest that very few Blanchard's Cricket Frogs live more than one year. The growth data for the Northern Cricket Frog was insufficient to support either semelparity or iteroparity. As a semelparous species, Blanchard's Cricket Frog may be susceptible to transient and temporary stressors that interfere with reproduction or recruitment.

Keywords. *Acris crepitans*, growth, lifespan, reproduction, semelparity.

Introduction

Blanchard's Cricket Frog (*Acris blanchardi*) and the Northern Cricket Frog (*Acris crepitans*) are small semi-aquatic hylids whose taxonomies are under debate (McCallum & Trauth, 2006, Rose et al., 2006, Gamble et al., 2008). Despite their wide-spread distribution and historically large populations, relatively little of their life history is known. In the face of current amphibian extinction and decline (Mendelson et al., 2006; McCallum, 2005; McCallum, 2007), it is valuable to resolve the missing links in each species' life history for developing conservation strategies (Bury, 2006). Because Blanchard's Cricket Frog is declining in the northern parts of its range (Reeder et al., 1998), abnormalities are becoming more prevalent in its populations (McCallum and Trauth, 2003a, b) simultaneous with abnormality increases (Wheeler et al., 2002, McCallum, 1999a) and die-offs (Trauth

et al., 2000) in other species residing in its range, and because the frog is at risk to predicted climate change (McCallum, 2010), it is especially important to resolve as much of its life history now while it is still common in parts of its range (McCallum and McCallum, 2006). Because the Northern Cricket Frog is closely related in phylogeny and habits, it is of interest to consider its ecology as well.

Much research on the life history of these frogs focused on color morph frequencies (Issacs, 1971; Nevo, 1973a; Gray, 1983) and inheritance (Pyburn, 1961), population ecology (Burkett, 1969; 1984), abnormality ratios (Reeder et al., 1998; McCallum and Trauth, 2003 a, b), pollution ecology (Russell et al., 2002; Theodorakas et al., 2006; McCallum and Trauth, 2007), and population declines (Beasley et al., 2005; Gray and Brown, 2005; Lehtinen and Skinner, 2006) and did not clearly indicate which species was involved. There is also evidence that Blanchard's Cricket Frog performs death feigning (McCallum, 1999b), may cannibalize its young (McCallum and Trauth, 2001), hibernates terrestrially (Irwin et al., 1999) and communally in gravel beds (McCallum and Trauth, 2003), and in cracked mud (Gray, 1971) upon exposure to sufficiently low temperatures (Brenner, 1969). There is some speculation that Blanchard's Cricket Frog is semelparous (Burkett, 1969; 1984). There is

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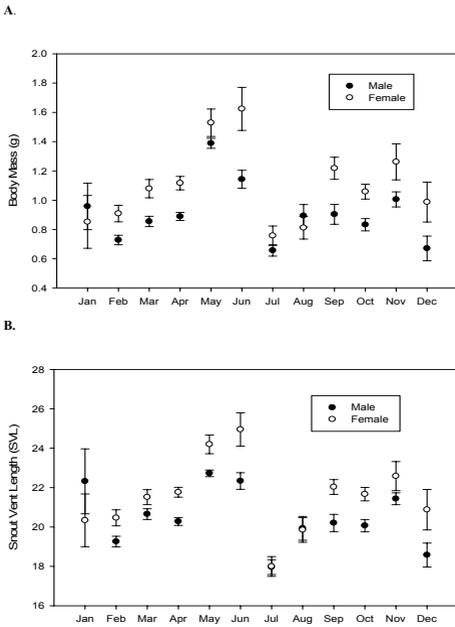


Figure 1. Monthly variation in body mass (A) and snout-vent length (B) of male and female *Acris crepitans*. The circle is the mean with the associated standard error bars.

little information available on the seasonal variation in growth and reproduction (e.g., clutch size, testicular and ovarian development) in either species. We collected a suite of natural history data on Blanchard's Cricket Frog and tested if it is semelparous. We also collected some seasonal size class information on the Northern Cricket Frog.

Body growth.—"Body condition is an important determinant of an individual's fitness, and its implications are of great interest to ecologists" (Green, 2001). Growth is one of the four primary aspects of life history relating to the health of an organism (Newman and Unger, 2002). Growth and body condition reflect the ability to get nourishment, resist stress, and store energy (Newman and Unger, 2002). Therefore, growth and seasonal size class variation provides important information for characterizing individual fitness and population health (Zera and Harshman, 2001).

Nevo (1973) provided ample evidence that variation of snout-vent length in *A. crepitans* and *A. blanchardi* is responsive to precipitation. So, characterization of seasonal growth requires examination of animals over a long period of time to control for the annual variation in temperature and rainfall. Tracking these small animals in the wild would be an arduous task

with their large populations, high activity and wide dispersal. Use of preserved museum specimens may be more practical. Each animal provides an instantaneous snout-vent length – body mass relationship for the date of collection. Consequently, size class data and extrapolated growth arising from museum specimens provides a useful baseline for future ecological comparisons. Semelparous organisms live only one reproductive season. If *A. blanchardi* or *A. crepitans* is semelparous, the population will have uniform size-classes in months when metamorphic individuals are not present (Schaffer, 1974).

Reproduction.—Trauth *et al.* (1990) reported that the clutch size of *A. blanchardi* ranged from 174-431 ($n=9$). *Acris crepitans* calls from April to August in Arkansas, eggs hatch in a few days and the froglets metamorphose after about 5-10 weeks (Trauth *et al.*, 2004). As is true of most common amphibians and reptiles (McCallum and McCallum, 2006), little is known about the male or female reproductive cycles of either *Acris crepitans* or *Acris blanchardi*. If *A. blanchardi* is semelparous we should see evidence (e.g., ovarian scarring) of former reproductive seasons in females.

Goals.—We attempt to characterize the growth and reproduction of *A. blanchardi* and establish if it is semelparous. We also collected seasonal size class information for *A. crepitans* and use that information to support whether it is semelparous. If either species is iteroparous, we should see individuals from multiple size classes throughout the year, skeletochronology should reveal multiple age classes in the early spring, and evidence of ovarian scarring should be present. Otherwise, the frogs must be semelparous.

Materials and Methods

Body growth.—Each specimen ($n = 1400$) one was positioned so that the angle running from the tip of the snout, through the spinal column and pelvic girdle to the anus closely approximated 180°. Then we measured the snout-vent lengths (SVL) with a standard metric vernier caliper. This measurement was the distance from the tip of the snout to the tip of the anus. We then opened the peritoneal cavity of each frog, drained the ethanol and padded it dry on a paper towel. We sexed each frog examination of the gonads. Next, we obtained the body masses (BM) with an AG 285 electric analytic balance (Mettler Toledo). We compared these data to published results for *A. blanchardi* from Nebraska and South Dakota (McCallum and Trauth, 2003, $n = 161$), Iowa (Johnson and Christiansen, 1976), and Kansas (Burkett, 1984), and to *Acris crepitans* from Georgia and Florida ($n = 95$; McCallum, 2003). Arkansas specimens came from the Arkansas State University Museum of Zoology herpetological Collection and specimens from Georgia and Florida came from the Georgia State

Table 1. Regional comparisons of *Acris crepitans* and *A. blanchardi* snout-vent length classes by month are presented as the ranges of the data about their means. Data is given as ranges for direct comparison to previously published papers in which ranges, not means and medians, were provided (The references in parentheses refer to the source manuscript for each data set). (Key: FL = Florida, GA = Georgia, NE = Nebraska, SD = South Dakota).

Month	<i>Acris blanchardi</i>										<i>Acris crepitans</i>	
	Arkansas (This study)		SD and NE (McCallum & Trauth 2003)		Iowa (Johnson & Christensen 1976)			Kansas (Burkett 1984)			GA and FL (McCallum 2003)	
	Males (mm)	Females (mm)	Males (mm)	Females (mm)	Males (mm)	Both (mm)	Females (mm)	Males (mm)	Both (mm)	Females (mm)	Males (mm)	Females (mm)
January	18-27	15-27										23
February	15-22	16-25									16-21	15-21
March	14-26	13-30							16-27		15-21	14-24
April	14-27	15-28		24-26	19-25		20-29	16-27		16-34	19-24	19-25
May	20-26	18-30	19-27	23-32	18-27		20-29	20-29		21-34	20-23.5	19-24
June	20-28	22-31	22-26	25-30	23-27		26-33	21-29		22-32	20-24	23-25
July	11-25	11-29	15-24	16-29	22-27		23-31	22-29	11-21	25-34	20	
August	12-22	15-27	19-26	19-26		11-19			11-18		12-22	22-26
September	12-27	15-28	16-25	17-26		12-26			11-26		20	
October	15-25	16-26				14-28			11-28		13-17	12-23
November	20-22	16-28							14-26			
December	18-22	18-23							19-26		11-21	

Museum herpetology collection.

We modeled the relationship between SVL and BM using non-linear regression with inclusion of the 95% confidence interval and the 95% prediction intervals. I modeled the monthly variation in size classes for both sexes using a One-Way Analysis of Variance (ANOVA). I evaluated investigator measuring error by measuring a single frog 30 times with vernier calipers and then calculating the mean and standard error for those measurements. I used regression analysis to verify if investigator error was stable.

Reproduction.—Sixty-five female and 114 male post-metamorphic *A. blanchardi* originated from Jane's Creek near Ravenden Springs (Randolph County, Arkansas) between 1 September 1999 and 1 January 2002 and came from the Arkansas State University Museum of Zoology (ASUMZ) herpetological collection. We used routine histological techniques to prepare the testes of males ($n = 10$ per month except Jan. = 2, Feb. = 12, Mar. and Sept. = 11) for light microscopy following standard histological methods (Presnell and Schreiber, 1997). Testes were dehydrated in a graded series of ethanol, cleared with xylene, infiltrated and embedded with paraffin, sectioned into serial ribbons (8 μ m in thickness), affixed to microscope slides using Haupt's adhesive, stained with Harris hematoxylin followed by eosin counterstaining (H&E), and mounted with coverslips. Maturation of sperm was categorized based on the presence of sperm, spermatids, secondary and primary spermatocytes, and spermatogonia. Sperm was designated as present if any one spermatid per histosection contained sperm. It was designated as absent if none of the spermatid cysts contained mature sperm. Germinal epithelium depth and spermatid cyst diameter were obtained from 15 histosections from each frog with an ocular micrometer.

Ovary samples came from 6 females each month except in the following months: January = 0, July = 7, August = 7, December = 3. Ovary images were captured with a Nikon DXM 1200 digital camera and either a Nikon Eclipse E600 epifluorescence Microscope and a Nikon SM2800 dissecting microscope. Maxi-

mum ovary length (OL), maximum ova diameter (OD), minimum ova diameter, and the diameter of mid-sized ova were determined using Nikon ACT-1 version 2.12 computer digitization software. Female snout-vent length (SVL) was recorded and used to calculate a gonadosomatic index (GSI) where $OL/SVL = GSI$ for each female. This was used instead of masses because of the small size of immature ovaries. Total numbers of mature ova and small vitellogenic ova were counted for each ovary possessing vitellogenic ova. Statistical analyses were performed using MiniTab 13.0 for Windows.

Lifespan.—Body size class data from above were analyzed to infer patterns supporting semelparity vs. iteroparity. Skeletochronology was used to validate these results with *A. blanchardi*. We obtained formalin (10%) fixed, ethanol (70%) preserved *Acris blanchardi* ($N = 21$) from MLM's research collection. The specimens originated from Bowie County (Texas, USA) between February-April 2005. The right femur of each specimen was removed and decalcified (Zug and Rand, 1987). Samples were dehydrated, infiltrated and then embedded in paraffin and histosectioned with a sliding microtome (Presnell and Schreiber, 1997). Histosections were mounted on glass slides, subjected to standard Hematoxylin-Eosin staining and affixed with cover slips (Presnell and Schreiber, 1997). Slides were observed using a Nikon Numarski DIC light microscope and the Lines of Arrested Growth (Lags) were used to assess age following Marnell (1998). We made three slides, each containing at least 5 histosections, for each frog.

Results

Body growth.—The estimate of investigator measuring error gave a mean SVL = 19.756 mm (SE = 0.001) with a range = 19.74-19.77 mm. Error remained stable from the first to the last measurement ($r^2 = 9.926$, $df = 29$, $P = 0.394$).

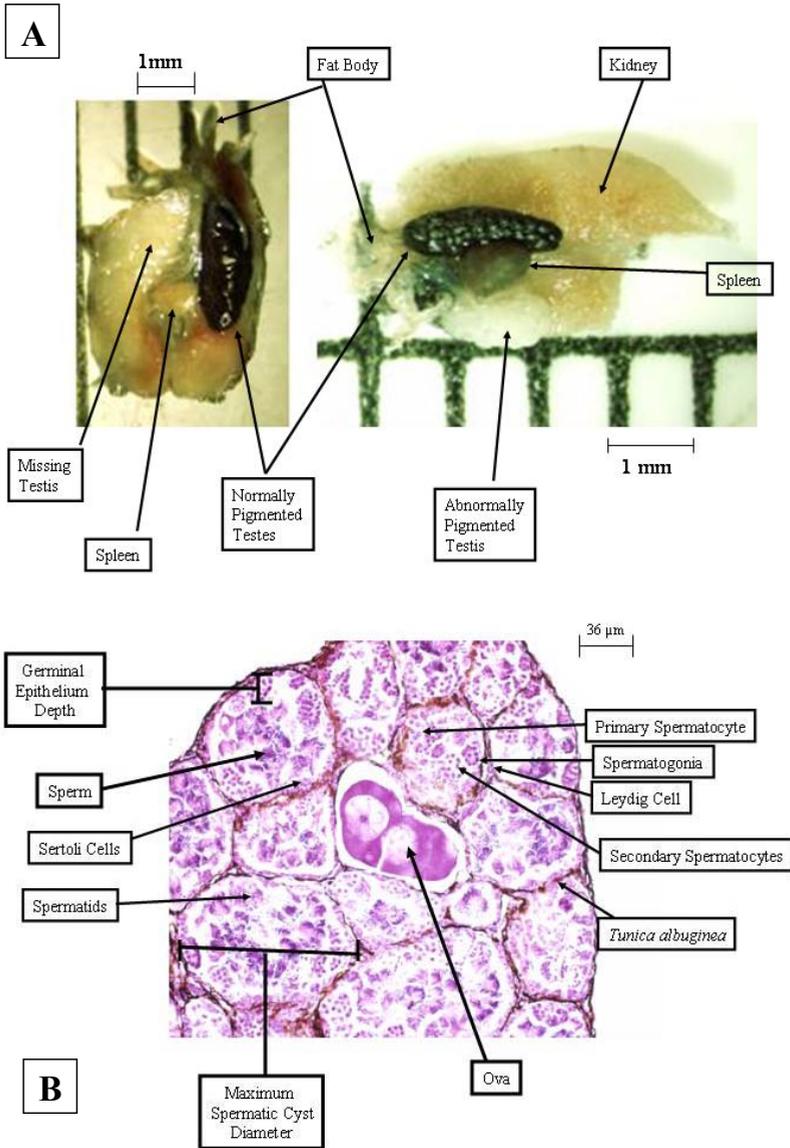


Figure 2. The male gross reproductive anatomy. A) Gross anatomy of the male urogenital tract. B) Histoanatomy of the testis in the Northern Cricket Frog (*A. crepitans*). Note abnormal instance of intersexuality. (Photos by M.L. McCallum).

The data ranges about their means are provided in Table 1 showing the monthly variation in SVL and BM for both species. Ranges are given instead of confidence intervals based on the availability of this information in the published literature. Female *A. blanchardi* BM (mean = 0.92 g, SD = 0.42, range = 0.09 – 2.52 g) and SVL (mean = 20.3 mm, SD = 3.1, range = 10.7 – 29.7 mm) best described by the polynomial regression equation ($r^2 = .840, P < 0.001$):

$$BM = 0.193950 - 0.0449324 SVL + 0.0029810 SVL^2 + 0.0000419 SVL^3.$$

The relationship between BM (mean = 1.06 g, SD = 0.60, range = 0.09 – 3.88 g) and SVL (mean = 21.2 mm, SD = 3.968, range = 10.7 – 31.7 mm) for *A. blanchardi* was best described by the quadratic regression equation (Fig. 1; $r^2 = 0.771, P < 0.001$):

$$BM = 0.560328 + 0.0213750 SVL + 0.0024595 SVL^2.$$

The SVL and BM of *A. blanchardi* varied by month throughout the year but the results were less conclusive for *A. crepitans* (Fig. 1). Metamorphic *A. blanchardi* first appeared in June with a large peak of newly

emerged juveniles appearing in July. These data follow anecdotal field observations (MLM pers. observ.). *Acris blanchardi* did not have uniform monthly variation in male snout-vent length ($F = 17.55$, $df = 746$, $P < 0.001$), male body mass ($F = 23.15$, $df = 746$, $P < 0.001$), female snout-vent length ($F = 13.73$, $df = 647$, $P < 0.001$), and female body mass ($F = 9.08$, $df = 647$, $P < 0.001$).

Male Reproduction in *Acris blanchardi*.—The macroanatomy of the urogenital system and the histoanatomy of the testis are shown in Fig. 2. Spermatic cysts with sperm present ($n = 1142$ from among 114 frogs) were significantly larger than those without sperm ($n = 493$ from among 114 frogs, ANOVA: $F = 362.27$, $df = 1$, $P < 0.001$). Spermatic cyst diameter was not constant throughout the year ($F = 362.27$, $df = 1$, $P < 0.001$, Table 1). A Tukey's multiple comparisons test (Tukey, 1959) revealed there were no significant differences among January, March, April, May, June, August, September, October, and November. Spermatic cyst diameter during January ($n = 2$, mean = $99.4 \mu\text{m}$, $SD = 0.015$) and March ($n = 11$, mean = $93.0 \mu\text{m}$, $SD = 0.028$) was significantly larger (95% CI = 0.002, 0.033) than February ($n = 12$, mean = $82.0 \mu\text{m}$, $SD = 0.020$). Diameters in December and February were

not significantly different, whereas July diameters had a significantly smaller spermatic cyst diameter ($n = 8$, mean = $55.0 \mu\text{m}$, $SD = 0.024$) than all other months. No trends for increasing spermatic cyst diameter were observed ($r^2 = 0.001$, $df = 1634$, $P < 0.167$).

The germinal epithelium depth (Table 1) was significantly smaller (ANOVA: $F = 404.64$, $df = 1634$, $P < 0.001$) in spermatic cysts with sperm ($n = 493$, mean = $37.0 \mu\text{m}$, $SD = 0.012$) than in those without sperm ($n = 1142$, mean = $21.0 \mu\text{m}$, $SD = 0.009$). Germinal depth varied significantly between months (Table 1) throughout the year, although no major trends were observed throughout the year ($r^2 = 0.021$, $df = 1634$, $P < 0.001$).

Sperm were present to varying degrees in all individuals during every month of the year (Table 1). Sperm were least common during March and August, whereas their presence was most conspicuous in January. Overall, sperm were present in 69 - 100% of the histosections from any one individual. The largest variance in sperm presence was recorded during October, and the lowest variance was observed during January.

Female Reproduction in *Acris blanchardi*.—The macroscopic gross reproductive anatomy for *A.*

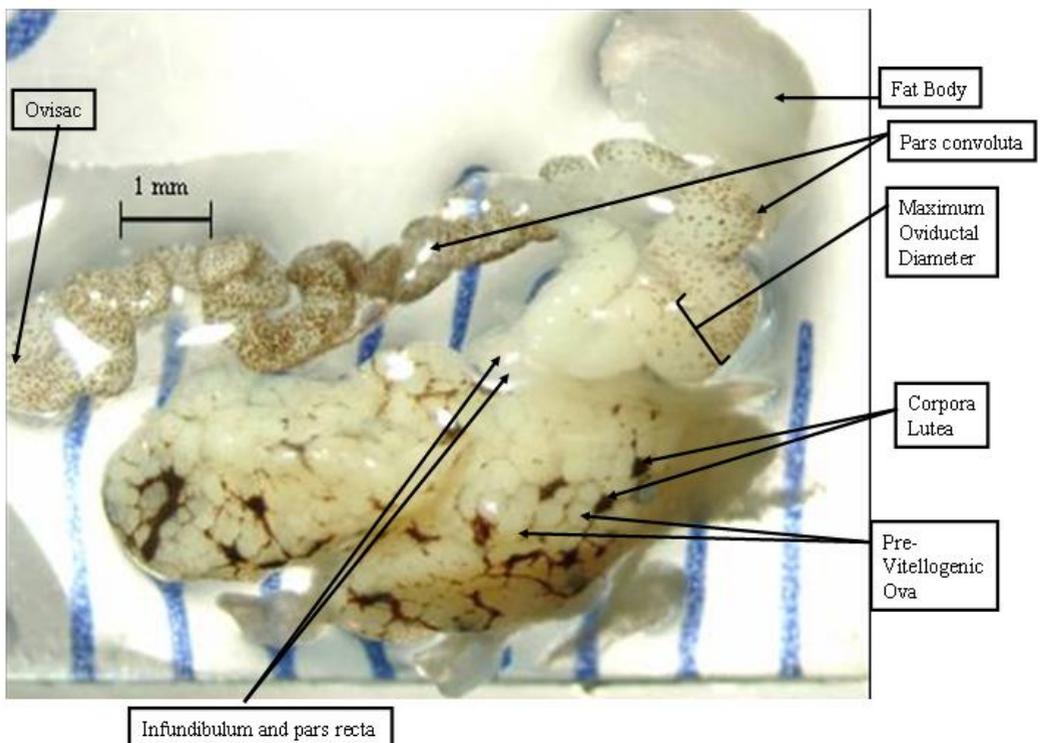


Figure 3. Female gross reproductive anatomy of the Northern Cricket Frog (*A. crepitans*). (Photo by M.L. McCallum).

Table 2. Reproductive condition of male and female *Acris crepitans* from northeast Arkansas. All linear measurements are in μm . Mn = number of males examined, Sn = total number of spermatid cysts measured, GED = mean epithelium depth, SCD = mean maximum spermatid cyst diameter, SD = standard deviation, Fn = number of females examined, OL = mean ovarian length, OD = mean oviductal diameter, SO = smallest ovum mean diameter, MO = medium sized ovum mean diameter, LO = maximum ovum mean diameter, Vn = number of ovaries with vitellogenic ova, Vit = number of vitellogenic ova present, %S = percent of spermatid cysts containing sperm.

Month	Mn	Sn	GED	SD	SCD	SD	Fn	OL	SD	OD	SD	SO	SD	MO	SD	LO	SD	Vn	Vit	SD	%S	SD
Jan	2	30	0.020	0.01	0.099	0.02	0	--	--	--	--	--	--	--	--	--	--	--	--	--	100	0.001
Feb	12	180	0.022	0.01	0.082	0.02	6	5.59	1.6	160.9	161	112.9	44	170.5	42	301.7	65	0	0	0	83	0.37
Mar	11	165	0.024	0.01	0.093	0.03	6	5.15	1.1	144.5	90	108.3	17	177	42	286	93	0	0	0	69	0.46
Apr	10	120	0.024	0.01	0.098	0.03	6	7.52	3.4	424.9	325	118.2	40	299.8	126	616.3	406	6	172	77	80	0.40
May	10	150	0.025	0.01	0.099	0.02	6	7.27	1.8	575.1	501	124.3	23	410	72	1070	351	11	220	110	72	0.45
June	10	150	0.020	0.01	0.091	0.02	6	10.82	5.0	787.9	427	109.8	29	410	169	940.5	431	9	233	50	86	0.35
July	10	120	0.020	0.01	0.055	0.02	7	4.95	2.7	248.1	397	78.8	32	164.4	160	399.5	542	4	220	104	39	0.49
Aug	10	150	0.026	0.01	0.092	0.04	7	5.57	2.8	447.5	440	82.4	32	185.7	160	373.3	389	2	214	86	60	0.49
Sept	11	165	0.022	0.01	0.096	0.02	6	3.86	1.4	0	0	82.8	21	146.8	41	259.3	50	0	0	0	84	0.37
Oct	10	150	0.032	0.01	0.088	0.02	6	4.87	1.6	128.5	217	105.3	17	212.1	36	306.6	68	0	0	0	47	0.50
Nov	10	135	0.023	0.01	0.094	0.02	6	4.68	1.1	93.8	158	101.5	26	210.5	60	334.5	95	0	0	0	77	0.42
Dec	10	120	0.029	0.01	0.081	0.02	3	4.22	1.2	67.9	118	99.2	5.8	205.2	44	402	104	0	0	0	54	0.50

blanchardi is shown in Fig. 3. The ovaries have two major lobes, a cranio-lateral lobe and a caudo-medial lobe. The distinction between these lobes is apparent in the immature ovary, but they become less distinct as the ova reach maturity. As with other anurans (Tyler, 2003), oviducts are paired, and tubular and divisible into an anterior infundibulum (ostium), pars recta, pars convoluta, and ovisac (Fig. 3).

Ovarian length varied significantly by month (ANOVA: $F = 3.70$, $df = 64$, $P = 0.001$; Table 2). Ovarian length was smallest in July (mean = 4.95 mm, $SD = 2.66$) and largest in June (mean = 10.82 mm, $SD = 4.98$). The GSI varied throughout the year with the largest value observed in June (Fig. 4). Vitellogenic ova were first observed in April (33%, 2/6 frogs), peaked in June (83.3%, 5/6 frogs) and July (85.7%, 6/7 frogs), and their presence extended through August (28.6%, 2/7 frogs). No vitellogenic ova were observed from September through March (Table 1). Individuals with longer ovaries and longer SVLs were most likely to possess vitellogenic ova ($z = 2.86$, $P = 0.004$; $z = 2.14$, $P = 0.033$, respectively). The total number of mature vitellogenic ova increased quadratically with increasing body length ($r^2 = 0.231$, $df = 31$, $P = 0.022$). The total number of vitellogenic ova (mature and immature combined) in each female was not closely associated with their snout-vent length ($r^2 = 0.079$, $df = 31$, $P = 0.119$). The size of the largest ova ($r^2 = 0.168$, $df = 60$, $P = 0.001$), of mid-sized ova ($r^2 = 0.266$, $df = 60$, $P < 0.001$), of the smallest ova ($r^2 = 0.149$, $df = 60$, $P = 0.002$), and maximum oviductal diameter ($r^2 = 0.136$, $df = 60$, $P = 0.003$) were positively related to SVL. Low regression coefficients suggest that other factors must also be contributing to

ovum and oviductal morphometrics. Snout-vent length was positively associated with ovarian length ($r^2 = 0.179$, $df = 60$, $P = 0.001$).

The largest ova ($r^2 = 0.612$, $df = 60$, $P < 0.001$), mid-sized ova ($r^2 = 0.571$, $df = 60$, $P < 0.001$), smallest ova ($r^2 = 0.249$, $df = 60$, $P < 0.001$), and maximum oviductal diameter ($r^2 = 0.580$, $df = 60$, $P < 0.001$) were positively related to maximum ovarian length. The largest ova (ANOVA: $F = 4.80$, $df = 64$, $P = 0.001$), mid-sized ova (ANOVA: $F = 4.83$, $df = 64$, $P < 0.001$), smallest ova (ANOVA: $F = 1.75$, $df = 64$, $P < 0.093$), and maximum oviductal diameter (ANOVA: $F = 3.94$, $df = 64$, $P = 0.002$) varied throughout the year (Table 2). Only one female (ASUMZ 24545) was found with oviductal eggs (right = 22 eggs, left = 31 eggs).

There was no significant difference among maximum ova diameters from July through March, a time period when the majority of ova were small. The largest diameters were observed between April and July (Table 2). Ovaries containing corpora lutea were observed in August ($n = 2$), October ($n = 1$), November ($n = 1$), and December ($n = 1$).

No vitellogenic ova were observed in ovaries collected from September through March. There was no significant difference in the number of premature vitellogenic ova from April through August (ANOVA: $F = 0.480$, $df = 31$, $P = 0.750$). There was also no significant difference among these months in the number of mature ova (ANOVA: $F = 0.200$, $df = 31$, $P = 0.939$). Lastly, there were no differences found among the months for total numbers of vitellogenic ova ($F = 0.460$, $df = 31$, $P = 0.764$).

Lifespan of Acris blanchardi.— Only 4/21 (19%) *A.*

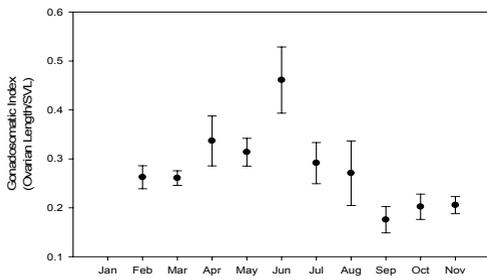


Figure 4. Monthly variation in the female gonadosomatic index (GSI). We calculated GSI by dividing snout-vent length by ovary length. No samples were available between December and January. The closed circle represents the mean and the respective standard error is shown as error bars.

blanchardi had LAGs. Only 1/21 (4.7%) frog survived two winters and could have participated in a second breeding season as an adult. All other cases had LAGs (3/21, 14.3%) positioned near the perieosteal surface of the femur suggesting these frogs were less than one year old.

Dietary observation.—Eighty percent (76/95) of *A. crepitans* from Georgia-Florida had food in their stomachs; most *A. blanchardi* appeared to have food in their stomachs, but the data for those were not quantified.

Discussion

Body growth.—This study is the first to characterize growth and seasonal size classes of *A. blanchardi* or *A. crepitans* throughout the entire year. Monthly variation in body size reveals that *A. blanchardi* populations in Arkansas begin producing metamorphs during June and continue through August. During the early spring, a few unusually large individuals are present (Fig. 2). These may represent individuals who survived a second winter hibernation.

Males and female *A. blanchardi* show a sexually dimorphic pattern of growth. Most male young-of-the-year reached adult size by November. Females grow quickly in the fall, but do not reach full adult size until May or June. The sexes first begin to diverge in body size during May. Body size dimorphism is more exaggerated in Nebraska-South Dakota than in Arkansas. This is probably a clinal relationship. Further, male and female *A. crepitans* are fairly similar in size.

Body size within this species is of interest because of its presumed taxonomic significance in discerning *A.*

blanchardi from *A. crepitans*. *Acris blanchardi* was described as being 2.5 times larger than *A. crepitans* (Harper, 1947). We did not see such dramatic differences in body sizes in this study.

Body size in this species varies across its range and among years based on precipitation levels (Nevo, 1973b) and larger frogs are more resistant to desiccation (Nevo, 1973b; Wilmer et al., 2000). During dry years, the animals respond by growing larger. Populations found in drier climates also grow larger in response to this stimulus. Since precipitation patterns across regions are graded from xeric in the North and the West to mesic in the Southeast, it is logical that this species' growth response should result in a cline following this rainfall pattern (Nevo, 1973b). However, our observations suggest that female body size is the most variable across the range of *A. blanchardi*; whereas, male body size is nearly uniform across the species range. This seems to indicate that males and females are unequally affected by hydric conditions, or that some other factor influences this dimorphism.

Female *A. blanchardi* in the Nebraska and South Dakota may be subject to additional evolutionary forces regarding body size. Trauth et al. (2004) suggested that this species might lay multiple clutches in Arkansas. One would predict that multiple clutching should only be adaptive where the breeding season is long enough to make producing second and third clutches advantageous. In regions such as the extreme northwest portion of this species' range, the breeding season is abbreviated as compared to the portion in Arkansas. If females from the southeast produce multiple clutches, they could take advantage of changing weather patterns throughout the summer. Larger frogs produce larger clutches (Lofts, 1974). The shortened breeding season in South Dakota and Nebraska may select against multiple smaller clutches leading to selection pressure for larger females that can lay larger single clutches each year. A larger body size would be necessary to accommodate the larger clutch size that is necessary to maintain fecundity potential in regions where multiple clutching does not occur. This might be an additional selective mechanism driving the geographic variation in female body size we have observed. Larger females should produce more eggs because of their increased energy reserves to fuel their production. These females would have larger single clutches than southeast animals, explaining the clinal differences in female size between regions. Males are not subjected to this additional evolutionary pressure, explaining why these differences in body size

are not as dramatic as those observed in females among regions.

Male Reproduction in Acris blanchardi.—We can deduce that males do not experience seasonal reproductive senescence because 1) sperm were observed in testes throughout the year, 2) spermatid cysts containing sperm were larger than those without sperm, 3) mean maximum spermatid cyst diameter remained relatively constant throughout the year, and 4) germinal epithelium depth was larger in spermatid cysts containing sperm than in those without sperm. The latter parameter also remained relatively constant throughout the year. Spermatid cyst diameter was lowest in July reflecting the dominance of immature metamorphs in the population during this month, which indicated that most breeding is probably completed by this point in time.

August spermatid cyst diameter is highly variable but stabilizes by September. This suggests that the reproductive systems of young-of-the-year male frogs quickly develop large spermatid cysts capable of reproduction despite their relatively small body size. Bayless (1969) suggested that young-of-the-year males might be capable of breeding within the same breeding season, but based his inferences on external morphologies. Although males are physiologically capable of breeding as early as their second month after metamorphosis, it is doubtful that these smaller, younger individuals can compete in the social structure of the chorus until the following summer. Moreover, few if any gravid females remain by the time metamorphs have reached maturity.

Female Reproduction in Acris blanchardi.—We found that as female SVL increased, so did the number of mature vitellogenic ova, vitellogenic ova, oviductal diameter, and the maximum ovum diameter. This indicated that larger females produce larger numbers of large eggs. Larger eggs are generally believed to possess more resources resulting in higher survivorship (Zera and Harshman, 2001) and, thus, provide large females with an adaptive advantage over small females. This might also explain why the female reproductive cycle in this frog is not continuous like the male. Attainment of a larger sex cell size is probably less critical for males and could even be maladaptive due to mobility constraints on large cell sizes. Larger females have larger ovaries, hence greater numbers of larger ova, which results in higher survivorship potential for their offspring (Zera and Harshman, 2001).

There are two female reproductive classes present beginning in June and lasting through July. By August, only the smaller size class is present. No individuals with corpora lutea are present after this time. This supports previous speculations that this is an annually-breeding species (Blair, 1961; Burkett, 1969; 1984; Pyburn, 1958). If most females survived a second year, either the two reproductive classes would persist through winter or only the larger size class would remain as the young-of-the-year mature. We would also expect corpora lutea (or corpora albicantia) to be more common than observed if females survived to breed a second season. Currently, investigations using skeletochronology to validate age classes are lacking for this species.

Although only one female was found with oviductal eggs, she was not in amplexus when collected. This provides some evidence that females might enter the breeding chorus prepared to immediately oviposit upon onset of amplexus. Whether the female is limited to laying the number of eggs already positioned in the oviduct, or if she is able to mobilize more eggs from the ovaries into the oviduct to manipulate clutch size among multiple males of varying quality is unknown and is a worthy area for further investigation.

Vitellogenesis appears to rapidly ensue during either late March or early April because no vitellogenic ova were observed in March even though both mature and immature vitellogenic ova were observed during April.

Timing of Reproduction in Acris blanchardi.—Histological data suggest that the initial onset of reproduction occurs sometime between late May and Early July with most egg laying occurring in early June. The enormous population surges along Arkansas streams due to emerging metamorphs (unpubl. data) support the histological data because metamorphosis is reported to occur at least 5 weeks after oviposition (Trauth *et al.*, 2004) placing most breeding in late May to early June. This is interesting because male *A. blanchardi* frequently begin calling as early as March and may continue into September throughout Arkansas. In southern parts of its range the species is known to breed in the spring and winter (Bayless, 1969) but only a single breeding period appears to occur in Arkansas. Why males continue to call for this duration is unclear and certainly leaves many evolutionary questions open for investigation. Clearly, more research on the geographic variation in the breeding ecology of this species is critical for a better understanding of how this species responds to environmental variables, especially given the impending global climate change.

Lifespan in Acris blanchardi.—Previous investigators speculated that this frog may be semelparous (Pyburn, 1958; Blair, 1961; Bayless, 1969; Burkett, 1969; 1984). Our size-class data suggests that the average body size follows a progression from metamorph to adult. During most months, the population composed of a nearly homogeneous assemblage of similar sized frogs. The only months where more than one age class is simultaneously present represent the overlap between breeding adults and metamorphosing juveniles. This dichotomy in body sizes quickly disappears suggesting that adults are leaving the population.

The female reproduction data may support semelparity. If numerous females were living multiple years, scarring on the ovaries from previous ovulations may be expected. Corpus lutea or corpus albicans were observed only in the fall. This may suggest that the ovaries recovered and scarring is not common, but in association with growth and skeletochronological evidence, it seems to support the semelparity hypothesis.

During the early spring, no metamorphs are present. If multiple age classes were present, skeletochronology would support this by revealing significant numbers of individuals with multiple LAGs. Our population did not demonstrate this. Only one individual was found with more than a single LAG. This individual must have survived two winters, making its age somewhere around 1.5 yr. These data suggest that *A. blanchardi* populations can be best characterized as semelparous.

Management Implications of Semelparity.—Because *A. blanchardi* appears to be semelparous it should be highly susceptible to extirpation from local habitats. If anything happens that significantly reduces reproduction or recruitment, the entire population at that location will succumb. *Acris blanchardi* may be especially susceptible to pathogens such as Chytrids, which attack newly metamorphosed frogs (Fellers et al., 2007). Additionally, semelparous organisms devote nearly all of their energy to reproduction leaving little to deal with stressors (Smith and Charnov, 2001). Therefore, mild environmental stressors may be much more serious during the breeding season.

Because few frogs persist more than one breeding season, the loss of a single reproductive season may extirpate this species. In a landscape where permanent and transient corridors are evident these populations may be recolonized. Evidence exists that these frogs do disperse reasonable distances between water bodies (Burkett, 1969; Gray, 1983; MLM Pers. Observ.). We speculate that accelerating wetland losses and increasing

implementation of flood control measures may disrupt the ability of this species to disperse between acceptable habitats. In regions where this frog is a conservation concern, it is important that corridors be maintained and populations be monitored. In instances where *A. blanchardi* is extirpated due to a transient, temporary stressor and dispersal corridors are no longer available, population restoration via repatriation may be a viable activity.

Acknowledgments. We thank Jerry L. Farris, Anne Grippio, Roger A. Buchanan, Roger Abernathy, Bruce Bury, Ray Saumure and Chris T. McAllister for commenting on early versions of this manuscript. We thank Benjamin A. Wheeler, Robyn Jordan, Robert Neal, Charles McDowell, Ben Ball, Richard Rauschenberger, Jamie L. McCallum, Kelly Irwin, Lisa Irwin, Kevin Eads, and others for comments, discussions and field help during this study. We further thank the Arkansas Game and Fish Commission for research permits. Funding was provided by Sigma Xi.

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