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## Reproductive Ecology of *Rhinophrynus dorsalis* (Anura: Rhinophrynidae) in México

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**ABSTRACT.**—For this study, we examined a large sample of museum specimens to ascertain the reproductive ecology of *Rhinophrynus dorsalis* (Mexican Burrowing Toad) in México. Seasonal aboveground activity was highest during May to August, which tracks monthly rainfall patterns across the region. Contrary to previous assertions, mean body sizes were similar between males and females but females attained larger maximum sizes. Male testis size was largest in May, tadpoles appeared in June, and juveniles were present in July, indicating that most breeding begins in May following the first heavy rains of the year. Few specimens possessed food in their stomachs suggesting that aboveground activity may be more devoted to reproduction than to foraging. The timing of reproduction was variable because most gravid females were encountered during May to August, but some were also present in October and January. Clutch size was estimated to range from 630 to 7,700 eggs, which was positively correlated with female body size. Size to sexual maturity in both males and females was reached in 8–9 mo after metamorphosis and at much smaller minimum and average sizes than previously reported. Adult body size was negatively related to latitude, such that the largest specimens were from the most southern latitudes, especially for males. The reproductive ecology of *R. dorsalis* resembles distantly related burrowing anurans more so than their close aquatic relatives in Pipidae.

General patterns of amphibian ecology and evolution can be inferred from studies into the life history of individual species. For example, descriptive studies of courtship have led to theoretical advances in sexual selection (Ryan et al., 1990) and social behavior (Wells, 1977). Furthermore, empirical experiments with a single species (Resetarits and Wilbur, 1989) have extended our understanding of the role that variable behaviors play in the evolution of life history, such as oviposition site choice (Resetarits, 1996). Data on life history strategies can be analyzed in a phylogenetic context to elucidate evolutionary patterns of reproductive diversity within a clade (Portik and Blackburn, 2016) or to estimate the ecophysiological conditions under which reproductive modes evolved globally (Lion et al., 2019). Data derived from multiple populations on how reproductive traits vary across geography have important implications for predicting how species will respond to environmental change (Blaustein et al., 2001) and for documenting differential selection pressures within species populations (Miaud et al., 1999). Also, patterns gleaned from life history investigations are increasingly recognized as indispensable to the development of ecologically relevant conservation strategies (Crump, 2015). Life history has implications ranging from evolutionary theory to conservation biology, yet we still lack information on the reproductive ecology of several widespread North American anurans. The need to fill this gap in knowledge has been intensified by the rapid rate and severity of recent amphibian declines (Cohen et al., 2019).

*Rhinophrynus dorsalis* Duméril and Bibron, 1841 (Mexican Burrowing Toad or Sapo Borracho) is the sole extant member of Rhinophrynidae, an ancient, monotypic anuran family originating in the late Jurassic period (Blackburn et al., 2019), that is sister to the aquatic clawed frogs (Pipidae) from South America and Africa (Frost, 2020). *Rhinophrynus dorsalis* is a distinctive fossorial frog with a red orange middorsal stripe and irregular yellow dorsolateral marks on a purple-to-black background skin coloration (Fouquette, 1969, 2005). This species possesses a particular suite of anatomical traits, including short legs, a

conical head, stout body, small eyes, loose skin, and the absence of a tympanum, some features of which it superficially shares with distantly related burrowing frogs in the families Brevicipitidae, Hemisotidae, and Myobatrachidae (Ford and Cannatella, 1993). Unique among anurans, however, is that *R. dorsalis* exhibits a distinctive method of tongue protrusion that has been posited to be an adaptation for subterranean feeding (Trueb and Gans, 1983). This species is distributed from extreme southern Texas (USA) along the coastal plain of the Caribbean versant through Belize south to Nicaragua, and along the Pacific versant from Guerrero (México) to Guatemala and Costa Rica (Campbell, 1998; Savage, 2002; Santos-Barrera et al., 2010). *Rhinophrynus dorsalis* inhabits mesic to arid forests of coastal lowlands from sea level up to 1,400 m elevation in areas that contain loose soil (Eisermann, 2017). This burrowing species spends most of its life underground (Fouquette, 2005) and is irregularly encountered by collectors, usually after bouts of rainfall when breeding aggregations form that sometimes last for just a few days (Stuart, 1961). Sexual maturity is assumed to be reached at body sizes ranging from 50 to 90 mm snout-vent length (SVL; Dodd, 2013) with characteristic anuran sexual-size dimorphism (SSD) where females are much larger than males (Savage, 2002). Breeding occurs after heavy rains in roadside ditches, small pools, and other ephemeral waterbodies, with one clutch size estimate based on Costa Rican populations that ranges from 2,000 to 8,000 eggs (Foster and McDiarmid, 1983).

*Rhinophrynus dorsalis* possesses one of the most unique combinations of traits among extant anurans, including a specialized feeding apparatus (Trueb and Gans, 1983), independently derived lack of tympanic middle ear (Pereyra et al., 2016), prolonged aestivation (up to 2 yr: Fouquette and Rossman, 1963), distinctive superficial mandibular musculature (Tyler, 1974), and idiosyncratic cranial anatomy (Trueb and Cannatella, 1982). Rhinophrynidae is allied to Pipidae (Cannatella, 2015); thus, any characteristics that it shares with fossorial frog families, such as Nasikabatrachidae (Zachariah et al., 2012), represent convergence. *Rhinophrynus dorsalis* has not received much attention despite representing an early branching lineage of Anura, and its potential contribution to the study of convergent evolution. Available life history information for *R.*

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*dorsalis* is limited to a few historical reports based on largely isolated breeding events across its extensive distribution (e.g., Orton, 1943; Starrett, 1960; Stuart, 1961; James, 1966). Because the reproductive ecology of *R. dorsalis* has not been examined in detail, we set out to use museum specimens to investigate life history variation in this species across México. Our densely sampled data set provides insight into reproduction of the only living member of an ancient anuran lineage and would not have been possible without preserved specimens in natural history museums.

#### MATERIALS AND METHODS

We measured 437 cataloged specimens of *R. dorsalis* from México housed in 6 museum collections: The University of Illinois Museum of Natural History (UIMNH;  $n = 200$ ) and the Illinois Natural History Survey (INHS;  $n = 5$ ), Champaign, Illinois; the Museum of Vertebrate Zoology (MVZ;  $n = 39$ ), Berkeley, California; the California Academy of Sciences (CAS;  $n = 99$ ), San Francisco, California; the Field Museum of Natural History (FMNH;  $n = 43$ ), Chicago, Illinois; and the Carnegie Museum of Natural History (CM;  $n = 51$ ), Pittsburgh, Pennsylvania (Appendix 1). The specimens were collected across 59 yr (1919–1978) from 8 states within the species' range in México (Fig. 1). We generated histograms using months as bins to compare seasonality in the number of specimen records of this data set with all *R. dorsalis* specimens from México on VertNet ([www.vertnet.org](http://www.vertnet.org)) and all Research Grade observations of *R. dorsalis* from México on iNaturalist ([www.inaturalist.org](http://www.inaturalist.org)).

We measured body size in snout–vent length (SVL) of all specimens to the nearest 0.01 mm using hand calipers. We distinguished recently metamorphosed frogs from tadpoles by the presence of forelimbs (Gosner stage 42), and from juveniles by the presence of a tail (Gosner stage 45; Gosner, 1960). We relied on direct examination of the gonads to assess sex because *R. dorsalis* does not exhibit the typical secondary sexual characteristics used to assign sex in adult frogs. For example, *R. dorsalis* lacks an external eardrum (tympanum), males lack enlarged (or cornified) thumb pads, and males lack external vocal sacs. The presence of enlarged (swollen) testes in combination with a larger body size was used to determine sexual maturity in males. We measured length and mid-width of the left testis from mature male specimens and estimated male fertility by calculating testis dimensions as a percentage of male SVL (Meshaka, 2001). To reduce some of the potential errors derived from preservation artefacts, all reproductive organs of male specimens were measured from the left side of the body (Lee, 1982).

We assigned maturity in females based on the following 4 ovarian stages: 1) oviducts were thin and uncoiled, and the ovaries were somewhat opaque; 2) oviducts were larger and more coiled, and the ovaries contained some pigmented oocytes; 3) oviducts were thick and heavily coiled, and the ovaries were in various stages of clutch development; and 4) oviducts were thick and heavily coiled, and the ovaries were full of polarized ova with few nonpolarized ova, which we used as evidence of a mature clutch and gravid female (Meshaka, 2001).

We estimated sexual size dimorphism (SSD) using the Lovich and Gibbons (1992) index by dividing the mean adult SVL of the larger sex (females) by the mean adult SVL of the smaller sex (males) and then subtracting 1. When the sexes are equal in size,  $SSD = 0$ ; when males are larger,  $SSD < 0$ , and  $SSD > 0$  when females are larger. We used this estimate of SSD to examine

geographic variation because this index is statistically optimal among available dimorphism indices (Smith, 1999).

We examined metabolic tradeoffs temporally between reproduction, feeding, and energy storage by calculating the monthly incidence of developed fat bodies, large liver sizes, and reproductive status (Lu et al., 2008). We assessed the extent of lipid deposits associated with gonads (i.e., fat bodies) in the body cavity of specimens based on 3 scores: 1) trace amounts or no fat bodies; 2) an intermediate volume of fat bodies; and 3) a high volume of fat bodies that extend anteriorly within the body cavity (Meshaka, 2001). We used the highest score as an estimate of the monthly incidence of extensive fat bodies relative to all males and females examined in each month. We recorded the number of individuals of each sex with food in the stomach or an obvious food item in the upper intestines as a proxy for the monthly frequency of individuals that had been feeding in each month. We also examined the extent of liver development into the visible posterior section of the body cavity based on a scoring system: 1) the liver had few lobes that occupied up to 30% of the body cavity; 2) the liver had several large lobes that occupied from 31% to 75% of the body cavity; and 3) the liver had many large lobes that extended posteriorly into the inguinal region to occupy 76% to 100% of the body cavity. It has been shown that liver mass in museum specimens is representative of livers from freshly collected animals (Withers and Hillman, 2001). We used the highest liver score to estimate the monthly incidence of well-developed livers relative to all males and females examined in each month because the liver plays an important role in energy storage, especially for species that aestivate for long periods (Mentino et al., 2017).

We used 12 randomly selected gravid females (ovarian stage 4) to examine clutch characteristics. We dissected the clutch out of the body cavity, gently removed excess moisture with a paper towel, and weighed the entire clutch to the nearest 0.01 g with an electronic scale. We weighed a subset of mature ova from each clutch and then counted the number of individual eggs in the subset. We extrapolated the mass of the counted subset of eggs to the weight of the entire brood to provide an estimate of clutch size (Giesing et al., 2011). We then weighed the female specimen with the clutch removed to generate estimates of relative clutch mass as per Shine (1980). We measured the diameters of 10 randomly chosen ova from each of the clutches using an ocular micrometer to the nearest 0.01 mm and used the largest ovum from each clutch to compare with clutch size and female body size.

The México state base map was obtained from d-maps.com and redrawn in Adobe Illustrator (Adobe Systems Inc., San Jose, California, USA). The photo of *R. dorsalis* was provided with permission by M. Pingleton. We used Excel 2016 (Microsoft Inc., Redmond, Washington, USA) and Program R version 4.0.3 (R Core Team, 2020) to organize data, conduct statistical analyses, and to generate quantitative graphics using the R package ggplot2 (Wickham, 2016). We present mean measurements followed by  $\pm 1$  standard deviation (SD). We checked for normality of data using Shapiro–Wilk tests, compared means between samples using 2-sample *t*-tests, compared variances with analysis of variance (ANOVA) *F*-tests and Levene's tests, and examined relationships between selected variables with Pearson's correlation coefficients. We recognized statistical significance at  $P < 0.05$ .

We wish to note that several specimens in our sample had various levels of damage to their anatomy, had been previously skeletonized, or had entire organ systems removed prior to our

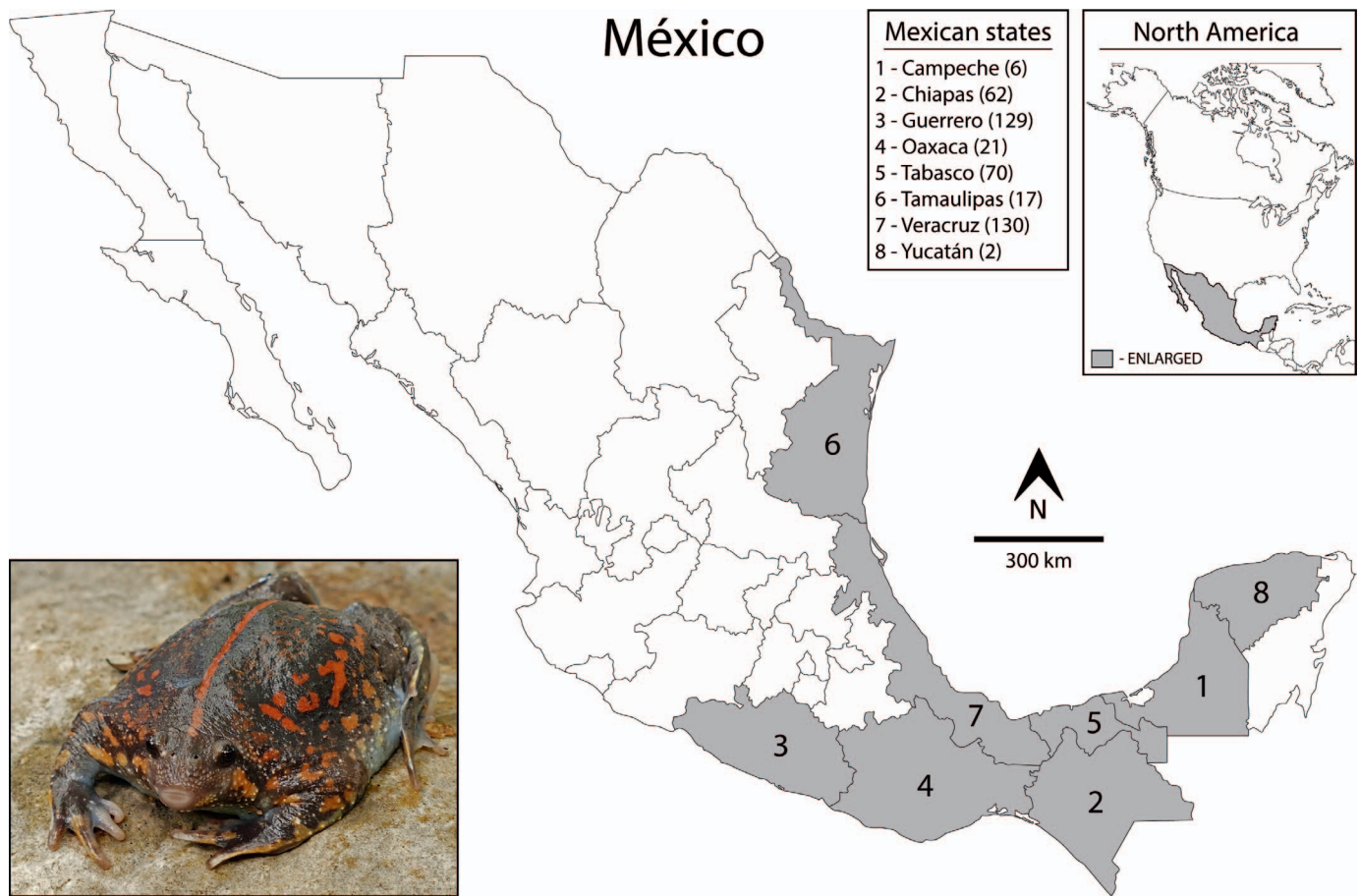


FIG. 1. Geographic origin of museum specimens in México of the Mexican Burrowing Toad (*Rhinophrynus dorsalis*) examined in this study. Sample sizes are given in parentheses after the Mexican state. Bottom left: Picture of *R. dorsalis* by M. Pingleton with permission.

study, so not all measurements were extracted from all specimens. Likewise, some records were incomplete as a result of nonsystematic data collection: 4.1% of specimens in our sample did not have any data on the month of their collection. Furthermore, a handful of specimens were cataloged under a single museum tag, such as large lots of tadpoles (e.g., 68 tadpoles cataloged as FMNH 191918). We chose to include data in relevant analyses and summary statistics from all specimens, including damaged specimens that still possessed interpretable characters or those that lacked specific collecting data, such as fat development when body size was unmeasurable or body size when collecting month was not recorded or seasonal activity when the specific locality was unavailable. Consequently, these inclusions influenced sample sizes across various categories of our analysis. The data underpinning these analyses are available from the authors upon request.

## RESULTS

**Monthly Frequency of Records.**—From VertNet collection records that span 148 yr (1857–2005) representing 724 specimens of *R. dorsalis* from México, we found that specimens were collected in every month of the year, with distinct peaks in June ( $n = 197$ ) and July ( $n = 201$ ), and fewer than 10 individuals in 4 separate months: February ( $n = 3$ ), October ( $n = 4$ ), November ( $n = 3$ ), and December ( $n = 4$ ; Fig. 2A). The sample of 437 cataloged specimens we examined generally reflected these same seasonal patterns (Fig. 2B). In our sample, specimens were collected

during every month except February and December; only one was collected in October (FMNH 105709), and just two in March (UIMNH 37223 and UIMNH 47873). The interval with the greatest incidence of captured individuals in our sample occurred during May–September, with an apparent peak in late summer (July–August). Monthly frequency of captures suggests activity peaks for different classes: males were captured most frequently in May, females from May to July, tadpoles in July, and juveniles in August (Fig. 2B). From community science observations on iNaturalist that span 16 yr (2004–2020) representing 158 Research Grade observations, we found that observations occurred in every month, with distinct peaks in June ( $n = 30$ ) and July ( $n = 35$ ), and fewer than 5 records in 2 separate months: February ( $n = 3$ ) and December ( $n = 2$ ; Fig. 2C). Taken together, the records from VertNet and iNaturalist corroborate the paucity of individuals in our sample that were collected in months outside of late-spring and summer, especially during September–April (Fig. 2).

**Body Sizes.**—The variance in body sizes (SVL) between males (variance = 78.61) and females (variance = 143.69) was not equal (Levene's test,  $F_{156} = 8.15$ ,  $P = 0.005$ ). The mean SVL of adult males ( $48.94 \pm 8.87$  mm, range = 30.6–71.47 mm,  $n = 118$ ) was not significantly different from that of adult females ( $48.57 \pm 11.99$  mm, range = 34.57–77.11 mm,  $n = 40$ ; 2-sample  $t$ -test with unequal variances,  $t_{54} = -0.19$ ,  $P = 0.85$ ; Fig. 3). The mean SVL of 266 juveniles was  $17.65 \pm 2.79$  mm (range = 13–23.25 mm). The mean total length of 62 tadpoles was  $33.23 \pm 4.88$  mm (range = 17.12–41.84 mm; body size  $14.46 \pm 4.97$  mm [range = 7.41–18.66 mm]; tail length:  $18.77 \pm 2.97$  mm [range = 9.71–23.55 mm]). The

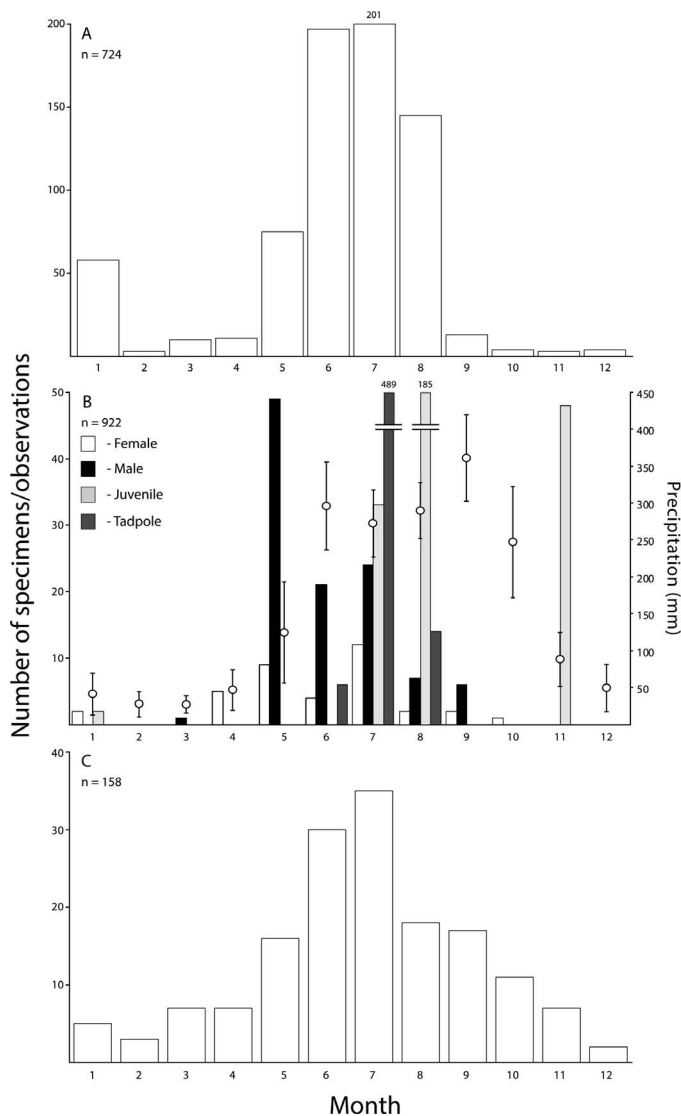


FIG. 2. Monthly incidence of Mexican Burrowing Toads (*Rhinophrynus dorsalis*) captures from México based on museum specimens and community science observations. (A) All specimen records available on VertNet that possessed month of capture data. (B) Specimens examined in this study separated by sex and life stage, which included 495 individual tadpoles (June and July) collected as lots cataloged under single specimen tags, and a series of 48 juveniles (November) cataloged in the same manner. Monthly rainfall (mean  $\pm$  standard error) displayed on the third axis. Precipitation values were taken from the Mexican states where 90% of our specimens were collected (Guerrero, Veracruz, Tabasco, and Chiapas). (C) All Research Grade observations (i.e., verified by an independent observer) recorded as on iNaturalist.

mean SVL for 2 specimens that had 4 legs and a tail was  $14.34 \pm 0.19$  mm (range = 14.20–14.47 mm). The SSD between adult females and adult males was marginally negative ( $-0.008$ ), indicating that the mean body sizes of the specimens were essentially equal.

**Testicular Cycle.**—Mean testis length as a percentage of SVL was  $11.95 \pm 2.38\%$  (range = 7.12–18.1%,  $n = 107$ ) and testis width was  $5.66 \pm 1.01\%$  (range = 3.57–8.6%,  $n = 107$ ). A seasonal change in testis dimensions was evident in our sample (ANOVA: testis length,  $F_{4,101} = 16.92$ ,  $P < 0.001$ ; testis width,  $F_{4,101} = 4.24$ ,  $P = 0.003$ ). Seasonal testis dimensions exhibited the greatest mean length and width in May, followed by a rapid drop in June, and

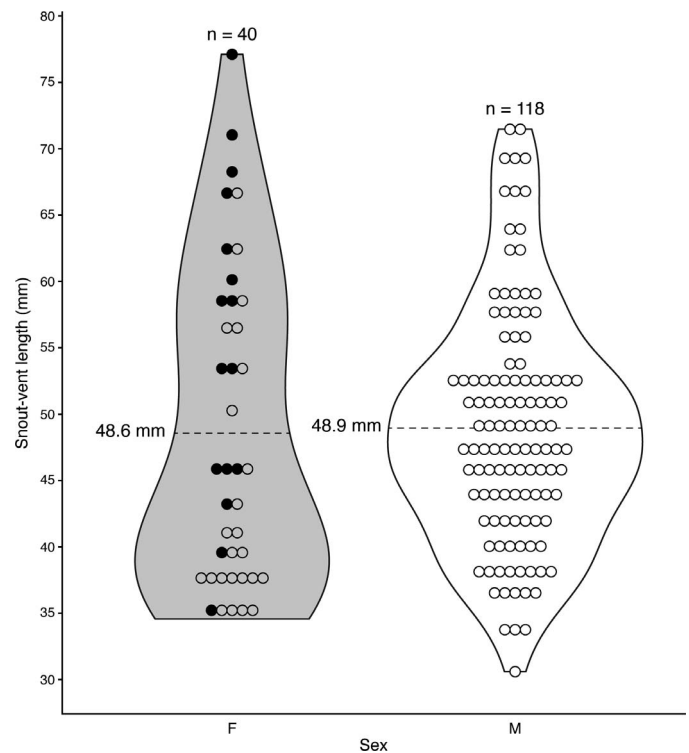


FIG. 3. Body size distributions of female and male Mexican Burrowing Toads (*Rhinophrynus dorsalis*) from México. Violin plots representing body sizes for all males and females in our sample. Sample sizes are presented above each plot. Dashed line indicates the sample mean. Filled circles represent gravid females (ovarian stage 4).

then relatively stable mean values through September, with the lowest mean length in July and lowest mean width in September (Fig. 4).

**Fat Deposits, Food Presence, Liver Size, and Reproduction.**—In general, males with food and extensive fat did not make up the majority (>50%) of specimens in any given month except for those with abundant fat bodies in May and August and food presence in September (Fig. 5A). The monthly percentage of males with extensive fat deposits was lowest during June (35%) and July (16%), and rapidly increased to a peak in August (71%). The monthly percentage of males containing food was lowest in August (14%) and highest in September (67%). Females, likewise, exhibited low percentages ( $\leq 50\%$ ) of specimens containing extensive fat and food in all months except for fat deposits in May (Fig. 5B). The monthly percentage of females with extensive fat development was highest in May (55%) and lowest in January, April, July, and August (all at 0%). The monthly percentage of females containing food was lowest in August (0%) and highest in January, July, and September (all at 50%). Large liver sizes were generally more common among males than females, ranging from 13% in July to 50% in March. For females, large liver sizes were present in January (100%), May (13%), and June (17%).

Fewer than 10% of ovarian stage 1 females had extensive fat deposits and none had well-developed livers (Fig. 5C). Females in ovarian stages 2 and 3 exhibited the highest frequency of food, but none of them exhibited extensive fat bodies. Large livers were detected at similar frequencies among females in ovarian stages 2, 3, and 4. The relatively higher percentage of food in the stomachs of stage 2 and 3 females, and a corresponding lack of extensive coelomic fat, indicates that feeding is more pronounced during these stages of clutch



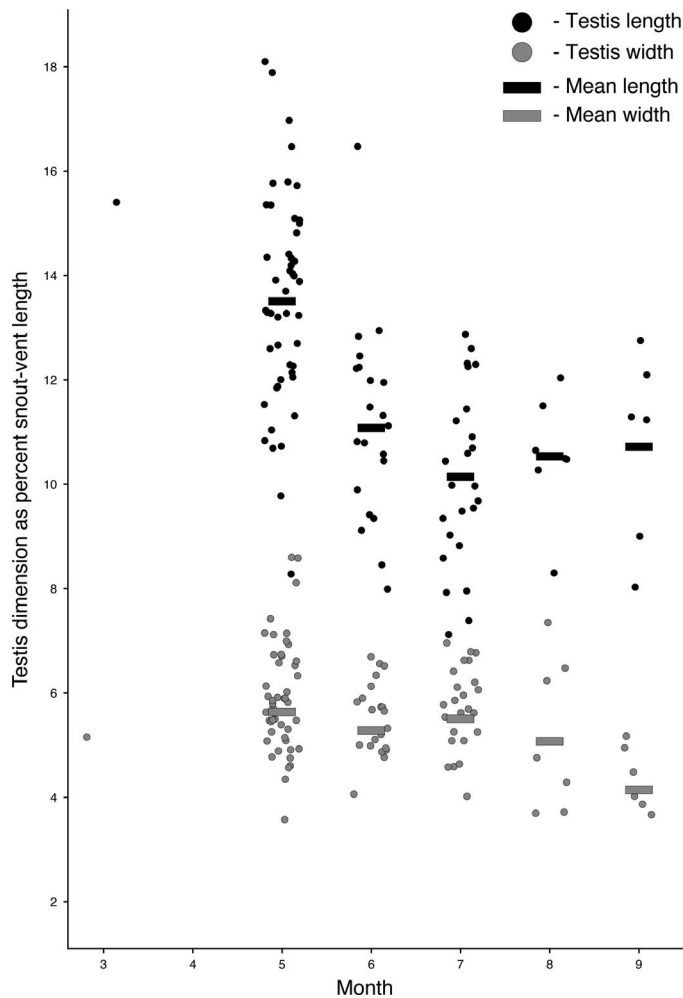


FIG. 4. Monthly distribution of testis length and width as a percentage of male body size in 107 Mexican Burrowing Toads (*Rhinophrynus dorsalis*) from México. Points are jittered for visual clarity between overlapping values.

development. The amount of ovarian stage 4 females with extensive fat suggests an increase of lipid stores prior to oviposition, which is corroborated by the high proportion of females with fat during the primary egg-laying season (May–June). The frequency of ovarian stage 1 females containing food was lowest (22%), followed by stage 4 (23%), stage 3 (33%), and stage 2 (50%).

**Ovarian Cycle.**—Gravid females (ovarian stage 4) were detected from May to August and in October and January (Fig. 6). The most gravid females, as the proportion of all specimens collected in a month, were found during May (78%) and June (83%), with the fewest in April (0%) and September (0%). The months with the lowest frequency of stage 4 females also exhibited the greatest frequency of stage 1 females (100% in both April and September). It is difficult to assess the ovarian cycle from the late autumn through spring because no specimens in our sample were collected in November, December, February, or March, but it appears that reproduction can occur in October and January. The time period with the most specimens (April to September) indicates that oviposition starts in May and generally is finished by August. To that end, all the tadpoles in our sample were collected from late June to mid-August: 26 June 1957 from Oaxaca (FMNH 121012); 3 July 1965 from Veracruz (INHS 30749);

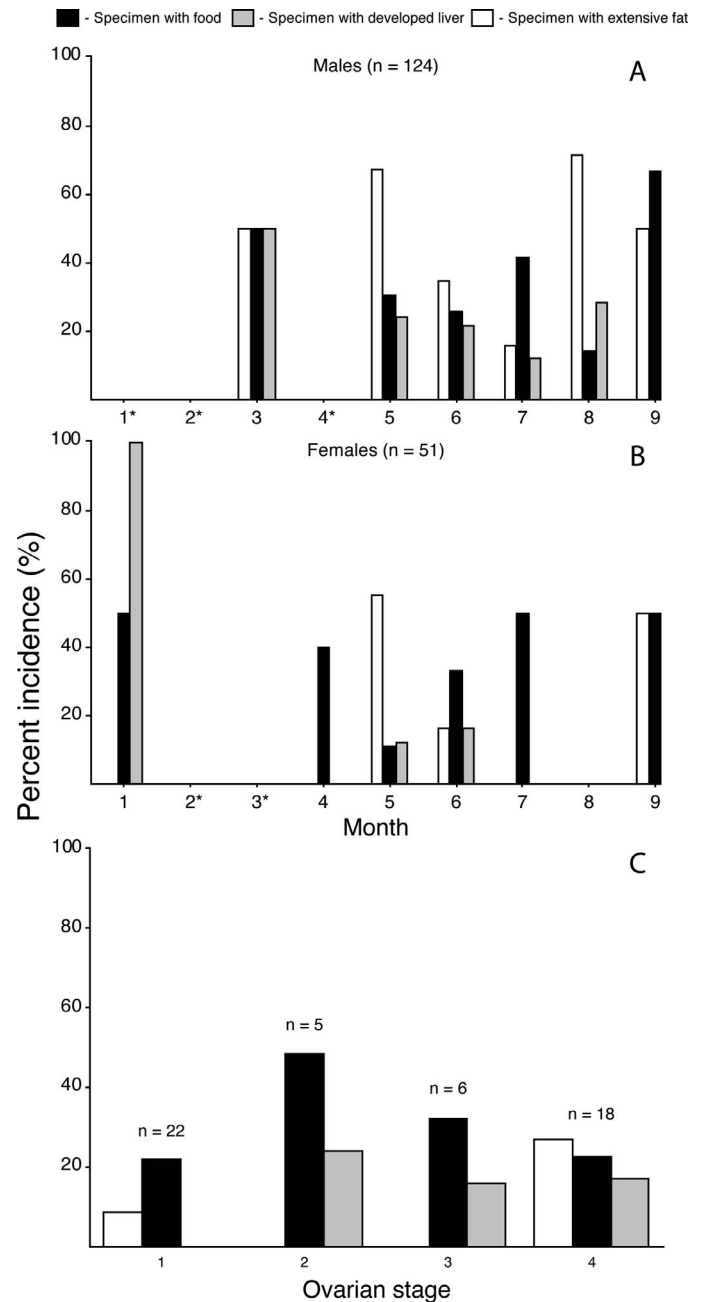


FIG. 5. Monthly frequency of food presence in the stomach, extensive fat in the body cavity, and well-developed livers in the Mexican Burrowing Toad (*Rhinophrynus dorsalis*) from México by male (A), female (B), and ovarian stage (C). Asterisks (\*) next to a month indicate that no specimens were available for examination.

7 July 1970 from Oaxaca (MVZ 92710; FMNH 171576, 191917–18); and 14 August 1955 from Veracruz (UIMNH 73482).

**Clutch and Egg Size.**—The clutch size, body size, and weight of gravid females were normally distributed (Shapiro–Wilk,  $W = 0.89–0.97$ ,  $P = 0.12–0.87$ ). From 12 gravid females (mean SVL =  $55.64 \pm 12.78$  mm, range = 34.57–77.11 mm; mean weight =  $26.01 \pm 15.81$  g, range = 8.2–56.7 g), mean clutch size was estimated at  $3,134.9 \pm 2,326$  eggs (range = 630–7,700 eggs). Clutch size was positively correlated with SVL (Pearson correlation,  $t_{10} = 7.23$ ,  $R = 0.92$  [0.72–0.98 95% CI],  $P < 0.001$ ) and specimen weight (Pearson correlation,  $t_{10} = 22.54$ ,  $R = 0.99$  [0.97–0.99 95% CI],  $P < 0.001$ ; Fig. 7). Clutch mass increased

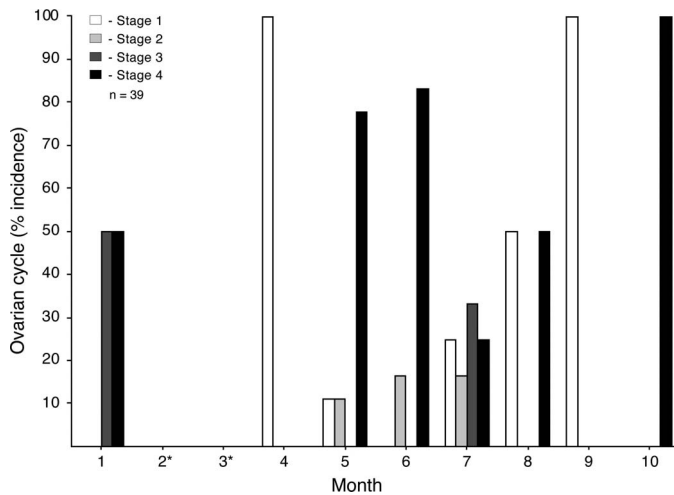


FIG. 6. Monthly ovarian cycle of female Mexican Burrowing Toads (*Rhinophrynus dorsalis*) from México. See text for details on reproductive stages.

significantly with increasing SVL (Pearson correlation,  $t_{10} = 8.43$ ,  $R = 0.94$  [0.78–0.98 95% CI],  $P < 0.001$ ). The relative clutch mass of these 12 female specimens was  $32.3 \pm 6.57\%$  (range = 20.18–41.67%) and was positively related to both SVL (Pearson correlation,  $t_{10} = 3.4$ ,  $R = 0.73$  [0.27–0.92 95% CI],  $P = 0.007$ ) and body weight (Pearson correlation,  $t_{10} = 3.26$ ,  $R = 0.72$  [0.25–

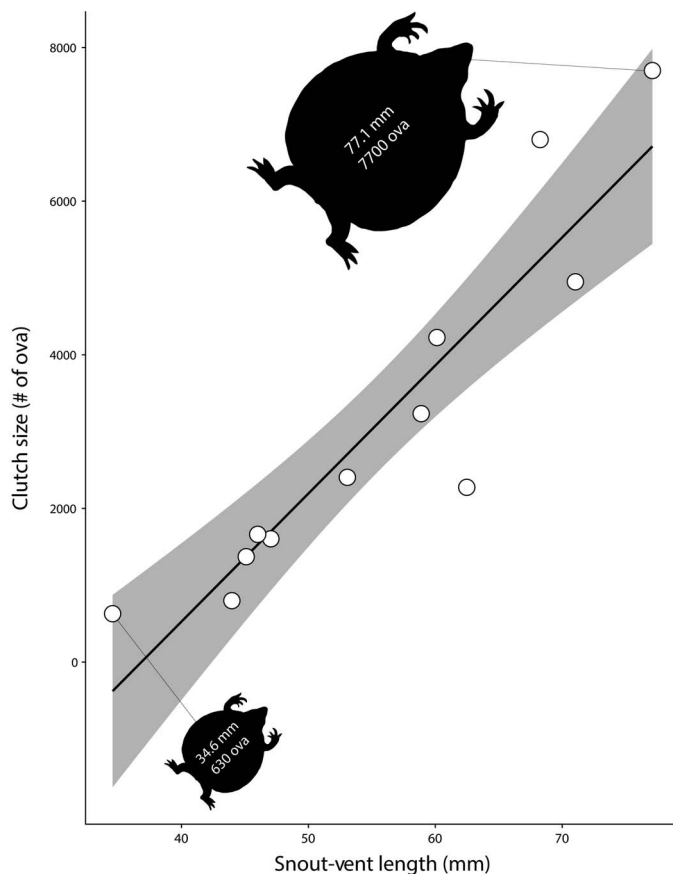


FIG. 7. The relationship between clutch size and body size in 12 female Mexican Burrowing Toads (*Rhinophrynus dorsalis*) from México. Grey shading indicates the 95% confidence interval. The equation for the line:  $y = -6,100 + 170x$ . Silhouettes modified from Eisermann (2017) and drawn to scale relative to each other.

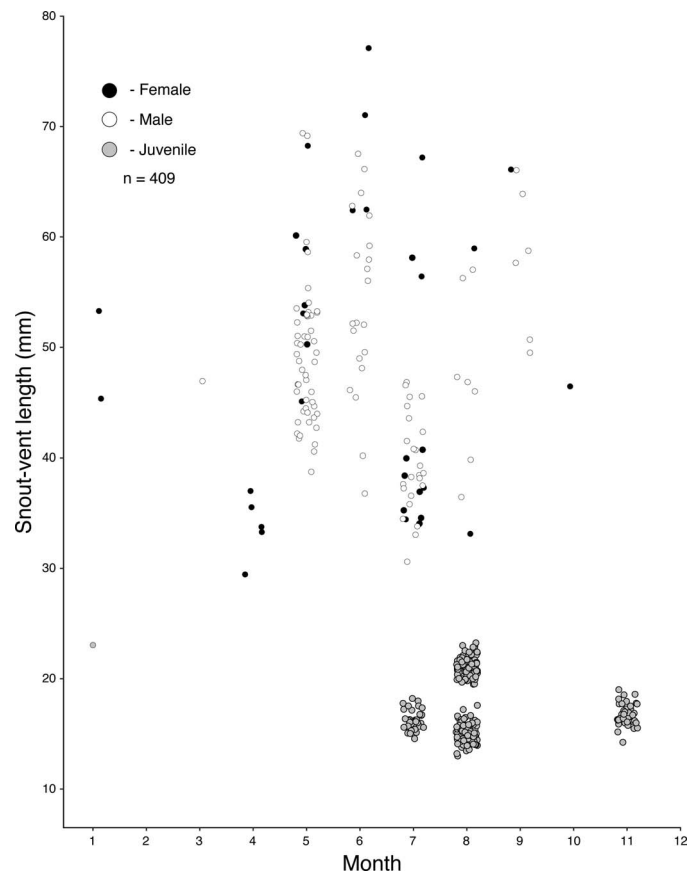


FIG. 8. Monthly distribution of body sizes for 36 females, 107 males, and 266 juveniles of the Mexican Burrowing Toad (*Rhinophrynus dorsalis*) from México. Points are jittered for visual clarity between overlapping values.

0.92 95% CI],  $P = 0.009$ ). The mean diameter of 120 measured ova from the same 12 gravid females was  $1.7 \pm 0.19$  mm (range = 1.07–2.44 mm). Mean and maximum ovum sizes were not correlated with body size ( $R = 0.31$ – $0.38$ ,  $P = 0.22$ – $0.33$ ), clutch size ( $R = 0.06$ – $0.15$ ,  $P = 0.64$ – $0.86$ ), or specimen weight ( $R = 0.11$ – $0.2$ ,  $P = 0.53$ – $0.74$ ).

**Growth and Sexual Maturity.**—Seasonally, the first tadpoles were collected on 26 June 1957 from Oaxaca (FMNH 121012) and the last on 14 August 1955 from Veracruz (UIMNH 73482), with the most collected in July ( $n = 489$ ; Fig. 2). The earliest seasonal juvenile record was a single individual (SVL = 25.27 mm) collected on 17 January 1978 in Guerrero (CAS 150150), and the last was a series of 48 juveniles (SVL range = 14.24–19.01 mm) collected on 2 November 1963 in Tabasco (CM 38925). During the most densely sampled monthly period (April–September), the first record of juveniles (SVL range = 14.57–18.21 mm) was a series ( $n = 33$ ) collected on 24 July 1974 (MVZ 112268–11230), and the last (SVL range = 13–17.59 mm) was a large series ( $n = 92$ ) collected on 14 August 1955 in Veracruz (UIMNH 73390–73481; Fig. 8).

The smallest adult male (SVL = 30.6 mm) collected on 20 July 1963 in Tabasco (UIMNH 62718) appeared to be sexually mature based on enlarged testes that were 8.82% the length of its body size. The smallest gravid female (stage 4) was 34.57 mm in SVL and collected on 1 July 1963 in Tamaulipas (UIMNH 64652), the smallest ovarian stage 3 female was 36.94 mm in SVL and collected on 20 July 1963 in Tabasco (UIMNH 62720), the smallest ovarian stage 2 female was 34.44 mm in SVL and

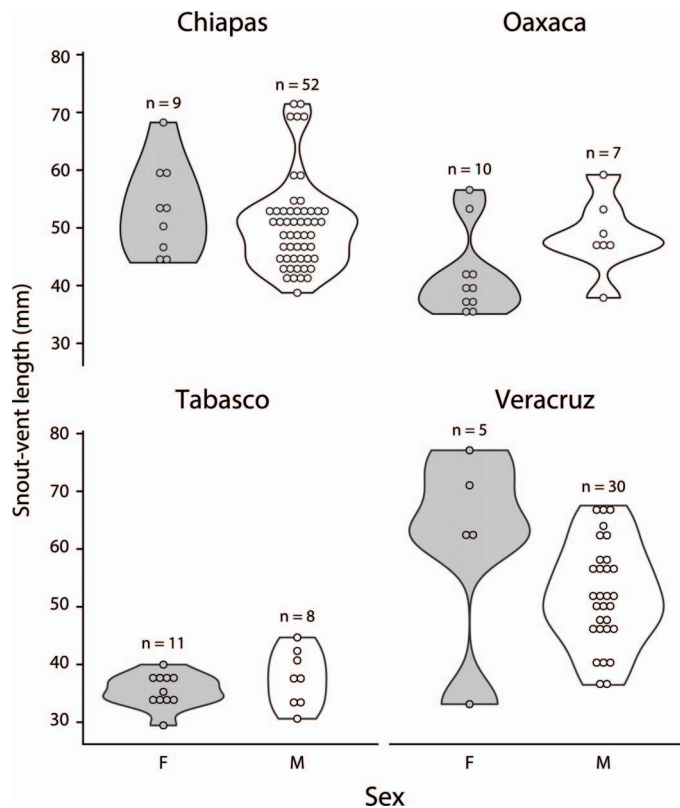


FIG. 9. Violin plots representing body sizes for males and females Mexican Burrowing Toads (*Rhinophrynus dorsalis*) across the 4 Mexican states that had  $\geq 5$  males and 5 females. Sample sizes are presented above each plot.

collected on 20 July 1963 in Tabasco (UIMNH 62717), and the smallest ovarian stage 1 female was 29.45 mm in SVL and collected on 15 April 1959 in Tabasco (UIMNH 47874). It is unclear whether the smallest ovarian stage 1 females were still maturing with egg laying to occur at larger sizes. Given that the smallest ovarian stage 4 female was  $< 5$  mm larger in SVL than the smallest ovarian stage 1 female, it is feasible that maturity and egg laying could have occurred at body sizes between 29 mm and 34 mm.

The mean SVL of ovarian stage 1 females was  $39.74 \pm 9.19$  mm (range = 29.45–66.11 mm,  $n = 20$ ), for ovarian stage 2 females it was  $50.89 \pm 12.03$  mm (range = 34.44–62.41 mm,  $n = 4$ ), for ovarian stage 3 females it was  $42.42 \pm 7.58$  mm (range = 36.94–56.57 mm,  $n = 6$ ), and for ovarian stage 4 females it was  $55.49 \pm 12.03$  mm (range = 34.57–77.11 mm,  $n = 16$ ). Mean body size of gravid females (stage 4) was significantly larger than that of all other ovarian stages (nongravid female stages 1–3 [ $41.76 \pm 9.73$  mm, range = 36.66–66.11 mm,  $n = 30$ ;  $t_{26} = 3.93$ ,  $P < 0.001$ ]).

Based on the seasonal distribution of body sizes and specimen occurrences, tadpole transformation time was approximately 2 mo (Figs. 2, 7). Males and females could have reached their minimum body size at sexual maturity (males = 30.6 mm, females = 34.57 mm) within 8–9 mo after metamorphosis and mean adult body size (males = 48.9 mm; females = 48.6 mm) approximately 12 mo postmetamorphic age.

**Geographic Variation in Adult Body Size.**—From the Mexican states that had adult specimens of both sexes, we selected those that had  $\geq 5$  adult females and 5 adult males to compare mean body sizes across geography, which resulted in 4 state comparisons. We found that the mean SVL of adult males was

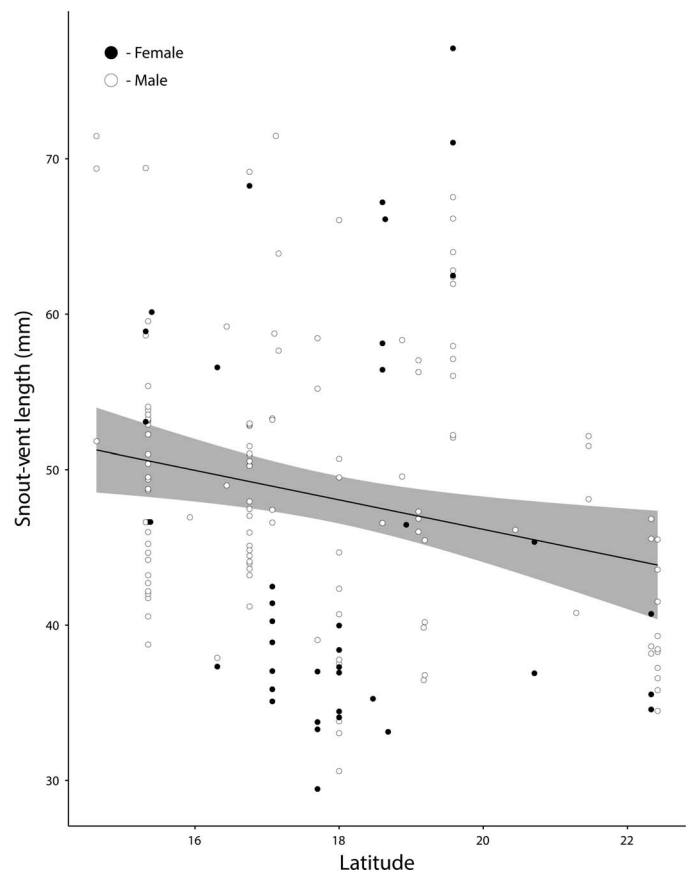


FIG. 10. The relationship between body size and latitude in the Mexican Burrowing Toad (*Rhinophrynus dorsalis*) from México ( $n = 163$ ). Grey shading indicates the 95% confidence interval. The equation for the line:  $y = 65.2 - 0.95x$ .

not significantly different from that of adult females in Chiapas ( $t_{11} = 0.98$ ,  $P = 0.34$ ), Oaxaca ( $t_{14} = -1.96$ ,  $P = 0.07$ ), Tabasco ( $t_{11} = -1.08$ ,  $P = 0.30$ ), or Veracruz ( $t_4 = 1.18$ ,  $P = 0.30$ ; Fig. 9).

Body size as SVL was negatively associated with latitude, with individuals from higher latitudes exhibiting smaller body sizes than those from lower latitudes (Pearson correlation,  $t_{161} = -2.7$ ,  $R = -0.21$  [−0.06 to −0.35 95% CI],  $P = 0.008$ ; Fig. 10). This latitudinal trend persisted when analyzing males separately (Pearson correlation,  $t_{116} = -3.04$ ,  $R = -0.27$  [−0.10 to −0.43 95% CI],  $P = 0.003$ ) but disappeared in an analysis of females only (Pearson correlation,  $t_{43} = -0.46$ ,  $R = -0.07$  [−0.36–0.23 95% CI],  $P = 0.65$ ). Adult body size showed no linear relationship to longitude (Pearson correlation,  $t_{161} = 0.42$ ,  $R = 0.03$  [−0.12–0.19 95% CI],  $P = 0.68$ ).

To further examine geographic variation in SSD, we grouped adult specimens by the degree of latitude or longitude of their collection (e.g., all male and female specimens from latitude  $15^\circ\text{N}$ ) that had  $\geq 2$  males and 2 females to calculate a mean SVL for each sex, which resulted in SSD estimates across 7 latitudinal degrees and 5 longitudinal degrees. We found that SSD was not significantly related to latitude (Pearson correlation,  $t_5 = -2.2$ ,  $R = -0.7$  [0.11 to −0.95 95% CI],  $P = 0.08$ ) or longitude (Pearson correlation,  $t_3 = 0.94$ ,  $R = 0.47$  [−0.7–0.96 95% CI],  $P = 0.42$ ).

## DISCUSSION

We used a robust sample of museum specimens from México to provide empirical data on several life history traits for *R.*



*dorsalis* that complement and, in some cases, extend previous findings. First, we wish to highlight general biases unique to studies based on museum collections that should be considered when interpreting their results (Robertson, 2008; Boakes et al., 2010; Wehi et al., 2012). Nondeliberate sex biases in vertebrate collections tend to be skewed toward males because they are often the more easily captured sex. In some animal groups, such as frogs, males exhibit a greater frequency of movements, more conspicuous visual displays, and audible calling to attract mates (Cooper et al., 2019). Breeding aggregations of *R. dorsalis* consist of mostly males, which conspicuously vocalize to attract females (Savage, 2002; Dodd, 2013), and we note that our sample contained over twice as many adult males ( $n = 124$ ) as adult females ( $n = 51$ ). Moreover, collectors' objectives vary through time, are dependent upon permits that differ across political boundaries, and generally follow assessments of species in need. *Rhinophrynus dorsalis* has not been in decline (International Union for Conservation of Nature Least Concern; Santos-Barrera et al., 2010) and thus was likely not a collecting priority during the well-documented declines of other Mexican anurans (e.g., Lips et al., 2004). We note that all the specimens in our sample were collected before 1980. Geographical biases are also widespread among museum collections such that the location and intensity of collecting are influenced by accessibility and species-specific differences in detectability (Reddy and Dávalos, 2003). *Rhinophrynus dorsalis* spends most of its life underground (Fouquette, 1969), rendering it difficult to target for collecting relative to surface-dwelling anurans. We attempted to address biases in museum collections by 1) focusing our study on a single political entity (México) to avoid changes in specimen collecting policies between nations; 2) examining a sample of specimens collected across decades and from multiple museums to minimize the impact of individual collector biases; and 3) including the largest sample of this species to date to generate a significantly robust biological signal to overcome these issues and even out potential collecting imbalances.

The literature on the reproductive biology for this species is based on a handful of primary data sources (Orton, 1943; Starrett, 1960; Stuart, 1961; Fouquette and Rossman, 1963; James, 1966; Nelson and Nickerson, 1966; Foster and McDiarmid, 1983; Sandoval et al., 2015; Stynoski and Sasa, 2018), which have been repeated verbatim, or with little added information, in regional synoptic guides (e.g., Lee, 1996; Campbell, 1998; Savage, 2002; Fouquette, 2005; Köhler, 2011; Dodd, 2013; Lemos-Espinal and Dixon, 2013; Lemos-Espinal et al., 2018). Most sources report that this species exhibits dramatic sexual size dimorphism (SSD) with much larger females than males (e.g., Fouquette, 1969). The degree of SSD in our sample was negligible between the mean adult body sizes of males and females, but there was evidence that females can attain larger maximum adult body sizes than males. The largest specimen we examined was a gravid female (SVL = 77.1 mm) from Veracruz (UIMNH 42654) and the largest male specimens (two at SVL = 71.5 mm) were from Chiapas (UIMNH 33585 and INHS 6572). The largest size record based on a physical specimen was 88 mm SVL for a female recorded by Nelson and Nickerson (1966) from Guatemala (MCZ A-2312). From Costa Rica, Savage (2002) reported an adult SVL range of 50–89 mm, with females reaching 89 mm and males only 75 mm, but there was not a clear basis for these measurements. Fouquette (1969) reported that adults reach sexual maturity at 60–65 mm SVL and that overall adult range is likely to be 50–88 mm, with females larger than males, which was echoed by Foster and McDiarmid (1983).

From the Mexican states of San Luis Potosí and Nuevo León, Lemos-Espinal et al. (2018; Lemos-Espinal and Dixon, 2013) reported that the maximum SVL of females is 88 mm and that adult males averaged 60–65 mm, values that were likely derived from Fouquette (1969). From the Yucatán Peninsula, Lee (1996) reported an adult SVL range of 60–65 mm SVL, and that females are substantially larger than males. Campbell (1998) reported from northern Guatemala, the Yucatán, and Belize, that males reach 65–75 mm in SVL and females 70–80 mm in SVL, but no reference material was mentioned for these size ranges. Despite seemingly widely repeated values for body sizes at maturity, few accounts referred to measurements from specimens or referenced wild individuals. From a recent account based on a single breeding night in Costa Rica, Sandoval et al. (2015) found that the mean body size of females (SVL = 81 mm,  $n = 15$ ) was larger than that of males (SVL = 72.1 mm,  $n = 21$ ), and that larger females were amplexed by larger males. It seems that the SSD in this species may be less extreme than previous reports have indicated because most accounts echoed values from Costa Rica (Savage, 2002) or Guatemala (Nelson and Nickerson, 1966). Nevertheless, females can exhibit larger maximum body sizes than males, and the SSD at a specific breeding site (e.g., Sandoval et al., 2015) is likely to be more prominent than when generalizing across its range. We suggest that the SSD in *R. dorsalis* may be most evident at tropical latitudes because body sizes were largest at the lowest latitudes in our sample and in the literature. However, this geographic pattern in body size was driven by a sex effect on the slope of the latitudinal cline, where the slope in males was steeper than that in females, such that males were smallest at northern latitudes and larger further south, whereas female body size did not change much across latitude.

From Costa Rica, Foster and McDiarmid (1983) reported the only clutch size estimate for this species (range = 2,000–8,000 eggs), which was repeated by Campbell (1998), Savage (2002), and Köhler (2011). We found that the clutch size range in México (630–7,700 eggs) conforms to the upper limit of this previous report but extends the lower estimate well below 2,000 eggs. In fact, we found 5 females with body sizes smaller than the mean of our sample (48.6 mm SVL) that were gravid, and these specimens had an average clutch size of 1,206 eggs (range 630–1,617 eggs). The smallest gravid female in our sample (SVL = 34.6 mm) was from Tamaulipas, indicating that either this species reaches sexual maturity at a much smaller body size in northern México than reported elsewhere or that the females south of México, from where most of the data in the literature originated, mature at larger body sizes. To that end, we found that adult body size in our sample was negatively related to latitude with larger specimens originating from southern locations. The largest specimen-backed body size for this species was recorded from Guatemala (Nelson and Nickerson, 1966), and all reports from south of México suggest that both sexes mature at larger body sizes than those we found (e.g., Foster and McDiarmid, 1983; Campbell, 1998; Savage, 2002; Sandoval et al., 2015). It remains unclear whether there is significant geographic variation in clutch size, and if so, whether it follows the observed latitudinal variation in body size.

In our sample, *R. dorsalis* was most frequently captured in late spring and summer, with very few records in autumn and winter. Additional specimen records from VertNet and community science observations from iNaturalist independently corroborated that *R. dorsalis* is active year-round in México and

that activity is most pronounced from May to August and lowest from September to April. We detected most gravid females from May to August, most tadpoles from June to August, and most juveniles during July and August, indicating that breeding generally begins in May after the first major rainfall and likely ceases by September. Males were also most common in May and their testis size was likewise largest in May and was much smaller in specimens collected during all other months. Nevertheless, we also found small juveniles in November and January, and gravid females in January and October, indicating that the timing of reproduction can be variable across its range. In Guerrero, Fouquette and Rossman (1963) collected a series of specimens during breeding aggregations that formed shortly after a storm on 19 July 1960. From the Yucatán Peninsula, Lee (1996) found tadpoles (Gosner stage 25; Gosner, 1960) in early October and suggested that reproduction may also occur later in the rainy season. In Nuevo León, Lemos-Espinal et al. (2018) suggested that this species will call and mate at any time during the year because of the unpredictability of rain and that tadpole development ranges from 1 to 3 mo. From Texas (USA), Fouquette (2005) also reported that this species will breed at any time of the year given adequate rainfall and that tadpoles take  $\geq 2$  mo to develop. From Guatemala, Stuart (1961) found tadpoles (mean body length = 18 mm) on 7 July that were at least 7-wk from the time of egg deposition, which was presumed to follow a heavy rainfall on 20 May, and that all tadpoles were metamorphosed by 17 August after reaching a maximum body size of 25 mm. Campbell (1998) reported that breeding occurs from June to September in Petén, Guatemala, perhaps based on Stuart (1961). From Costa Rica, Foster and McDiarmid (1983) reported that the populations breed once in late May or early June, and that all the tadpoles will have metamorphosed by the latter part of July and will disperse synchronously from wetlands.

Fecundity characteristics are poorly characterized among most fossorial anurans, but for those species with data, most tend to reach sexual maturity rapidly, invest a significant amount of their body weight to reproduction, and have short lifespans (Sullivan and Fernandez, 1999). Our findings on relative clutch mass (RCM) in *R. dorsalis* (range = 20–42%) are similar to those reported for other fossorial species (Székely et al., 2018), but at the higher end of the range for anurans in general (Kuramoto, 1978). Measurements from fresh *R. dorsalis* would likely exhibit higher RCMs because long-term preservation in ethanol undoubtedly reduced the weight measurements in our museum specimens (Deichmann et al., 2009). From a Neotropical frog community consisting of 16 species, RCMs ranged from 5.5% to 18% with a semifossorial species (Microhylidae: *Elachistocleis bicolor*) exhibiting the highest RCM values (Prado and Haddad, 2005). In a separate study on *E. bicolor* from Uruguay, Elgue and Maneyro (2017) found that the largest females produced the largest clutches, but RCM did not increase with increasing body size. In *Dermatonotus muelleri* (66.1–81.9 mm SVL), a fossorial microhylid from the Gran Chaco region in Argentina, RCM averaged 30.2% and was negatively related to SVL, with larger individuals investing less in reproduction in terms of clutch mass (Stănescu et al., 2016). Two small Australian frogs that differ in their habits have some of the highest reported RCM values: an arboreal species (Pelodyadidae: *Litoria dentata*) exhibited an RCM range of 34–63% (SVL range = 38–46 mm), and RCM did not increase with increasing SVL (Greer and Mills, 1998); and a terrestrial species (Myobatrachidae: *Crinia signifera*) exhibited an RCM range of 11–68%

(SVL range = 19–27 mm), and RCM was positively related to SVL (Lemckert and Shine, 1993). We found that reproductive effort increased with body size in *R. dorsalis*, such that the largest female (SVL = 77.1 mm) exhibited the highest RCM (42%), while the smallest female (SVL = 34.6 mm) had the second lowest RCM (22%). Interestingly, Lemckert and Shine (1993) found that female *C. signifera* with RCMs >35% were much less likely to be recaptured again, suggesting that frogs with greater clutch masses relative to their body masses were subjected to greater mortality.

The paucity of specimens with recent food items in their stomach, all of which were collected aboveground, was somewhat unanticipated given that prior accounts indicated that this species surfaces at night to feed (e.g., Foster and McDiarmid, 1983). The morphological study of Trueb and Gans (1983), however, posited that *R. dorsalis* has anatomical adaptations to feed underground. The long and narrow snout of this species is distinctive among all frogs because it has a spiny (keratinous) epithelium, calloused nose, and mandibular tip, which would facilitate forward penetration (Trueb and Cannatella, 1982; Trueb and Gans, 1983). The fundamentally distinct lingual mechanism of *R. dorsalis* appears well-adapted for subterranean foraging in narrow tunnels (Trueb and Gans, 1983), and its diet, reported to date, consists of only termites and ants (Savage, 2002; Dodd, 2013). The general lack of food in the stomachs among the specimens we examined provides corroborative evidence that most foraging in this species is accomplished underground and that terrestrial activities may be more limited than previously appreciated.

Temporal variation in the frequency of large liver sizes and extensive fat deposits suggests metabolic tradeoffs in relation to reproduction (Singh and Sinha, 1989). All nonreproductive females (ovarian stage 1) lacked large livers, and few had extensive fat, suggesting that their metabolism was geared toward utilization rather than storage. Gravid females (ovarian stage 4) tended to possess a combination of large fat bodies and large livers, indicating that metabolic substrates were maximal prior to breeding and that body lipids were likely mobilized for the production of ova (Fitzpatrick, 1976). Metabolic stores from lipids in coelomic fat bodies and glycogen in livers likely buffers *R. dorsalis* against unpredictable rainfall and may also be a byproduct of a species whose ecological niche is predominately fossorial. A comparative study on metabolic expenditure (lipid and nonlipid substrates) and energy allocation in relation to reproduction among fossorial, semifossorial, and nonfossorial anurans would be a fruitful avenue of future research (e.g., Long, 1986, 1987).

From a large sample of museum specimens, we found that *R. dorsalis* in México reproduces at much smaller body sizes than previously reported, and that geographic variation in body size exhibits a negative relationship with latitude, especially for males. Reproductive effort was high among gravid *R. dorsalis* relative to values reported for other anurans, with clutch masses comprising up to 42% of body weight, and larger females possessing larger clutches in terms of both weight and number of ova. The typical anuran female-biased SSD was nonexistent in our sample, suggesting that *R. dorsalis* may differ in this aspect from species with a similar adult morphology (Zachariah et al., 2012). This species does not appear to forage much while aboveground because few adult specimens contained evidence of recent food items. Comprehensive reproductive information from the northern (Texas) and southern (Costa Rica) ends of its

range will shed much needed light on the extent to which this species varies in life history characteristics geographically.

The detailed life history information we gleaned for an understudied, ancient lineage of anuran would not have been possible without museum collections. Empirical knowledge of how organisms live (i.e., natural history) is essential for understanding how environmental changes affect biodiversity (Tewksbury et al., 2014) and for conserving species (Greene, 2005). Natural history collections, in fact, house the specimen resources needed for documenting essential biodiversity variables (Kissling et al., 2018), yet they are underutilized in this endeavor (Winker, 2004). The careful examination of museum specimens can also lead to discoveries that would not have been possible from other lines of evidence (e.g., Campbell et al., 2018) because specimens are one of the only sources of biodiversity data that contain information as varied as diet, reproduction, and morphology for a single organism (Meineke et al., 2019). Museum specimens were vital to detailing the reproductive ecology of the poorly known *R. dorsalis* and they will continue to be central to understanding the past, present, and future of biodiversity.

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#### APPENDIX 1

*Specimens Examined.*—All specimens from México. CAS: California Academy of Sciences; CM: Carnegie Museum of Natural History; FMNH: Field Museum of Natural History; INHS: Illinois Natural History Survey; MVZ: Museum of Vertebrate Zoology; UIMNH: University of Illinois Museum of Natural History.

*Rhinophrynus dorsalis*: CAMPECHE: CAS 145821, FMNH 105709, MVZ 164755–164758. CHIAPAS: FMNH 117985–117988, FMNH 122285–122290, FMNH 122292–122298, FMNH 122300–122305, FMNH 173958, INHS 6572, UIMNH 11207–11222, UIMNH 11224–11235, UIMNH 32507–35212, UIMNH 33585–33587. GUERRERO: CAS 138045–138046, CAS 142492–142583, CAS 150150, MVZ 112268–112300, MVZ 117606. OAXACA: CM 157128, FMNH 105132–105133,

FMNH 105416–105422, FMNH 121012, FMNH 171576, FMNH 191917–191918, MVZ 51750, UIMNH 32504–32506, UIMNH 37223, UIMNH 42672–42673. TABASCO: CM 40078, CM 38925a–38925z, CM 38925aa–38925vv, UIMNH 47873–47879, UIMNH 62714–62727. TAMAULIPAS: CM 90105, UIMNH 32500, UIMNH 64640–64654. VERACRUZ: CAS 71765–71767, FMNH 1907, FMNH 208013–208014,

INHS 28075–28076, INHS 28176, INHS 30749, UIMNH 26533–26537, UIMNH 32501–32503, UIMNH 42653–42654, UIMNH 42656–42661, UIMNH 42663–42664, UIMNH 42666, UIMNH 42668–42671, UIMNH 49279–49280, UIMNH 64655, UIMNH 73389–73482. YUCATÁN: FMNH 551, FMNH 153417.