Characterization of Thermal and Hydric Conditions of Nesting Sites of Female *Aspidoscelis costatus costatus* (Squamata: Teiidae)

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Abstract.—**Reproductive success in oviparous squamates requires the selection of an appropriate nest site for oviposition. The resulting environmental conditions directly influence different biological traits of offspring. We studied thermal and hydric conditions of nesting sites before, during, and after the egg-laying season of *Aspidoscelis costatus costatus* (Balsas Basin Whiptail) in central Mexico to assess their respective relationships with the female reproductive cycle. We placed electronic thermometers directly into nests and measured soil humidity using a modification of the McGehee method. We measured and classified 45 nests of this subspecies, all located under volcanic rocks, as either active (i.e., with developing turgid, white-shelled eggs or shell remnants) or inactive (i.e., those from previous reproductive seasons as indicated by brown eggshells). We located active nests between June and September, whereas the inactive nests were found throughout the year. The average temperature and humidity in nests were 24.6° C and 19.5%, respectively, during the nesting season; however, neither temperature or humidity was optimal for clutch development in the months before and after the observed nesting season. Our study suggests that gravid female *A. c. costatus* respond to environmental seasonal cues for oviposition (obtained from nesting sites) that coincidentally influence completion of embryonic development. This is the first study of nesting sites in *Aspidoscelis* that could be monitored without destruction of the nest. Knowledge of the location of nesting sites for this species has major implications for its conservation, especially in patches of suitable habitat in a crowded urban landscape.**

Key Words.—Balsas Basin Whiptail; high-elevation; incubation; México; nesting; soil humidity; temperature

Introduction

Approximately 80% of squamate species are oviparous and they generally do not exhibit parental care (Pianka and Vitt 2003; Vitt and Caldwell 2009). Thus, embryonic development and survival after oviposition are dependent on the incubation environment and nest site-selection of females (Thompson et al. 1996). Nesting site selection in oviparous lizards represents the ultimate test of the fitness of an individual, given the persistence of a mode of existence that does not require parental care.

The incubation environment in a nest is defined by both biotic and abiotic factors, with temperature and humidity playing the predominant roles (Calderón-Espinosa et al. 2018; Li et al. 2018; Pruett et al. 2020).

Physiological, morphological, and ecological traits of the hatchlings are in part temperature-dependent (Andrewartha et al. 2010; Esquerré et al. 2014). Additionally, the temperature within a nest influences the developmental rate and the incubation time of the embryos (Andrews et al. 2000; Parker and Andrews 2007). Moreover, the hydric environment can affect the usage rate of yolk, calcium integrity of the shell, duration of the incubation period, hatching rate, and mass of the offspring (Ackerman and Lott 2004).

In lizards of the genus *Aspidoscelis* (Teiidae), cyclic reproduction seems to be common in seasonal environments (Hernández-Gallegos et al. 2003; Manríquez-Morán et al. 2005; López-Moreno et al. 2016) and may continue as long as required resources are available (Mojica et al. 2003). Diversity in the

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reproductive patterns (e.g., both non-cyclic and cyclic; both oviparity and viviparity), however, suggests the absence of group-wide genetic factors responsible for the regulation of the reproductive activity (Fitch 1985). For example, reproductive activity in squamates can be influenced by rainfall (Colli 1991), temperature (Dearing and Schall 1994; Hernández-Gallegos et al. 2003; Granados-González et al. 2015), photoperiod (Rodríguez-Ramírez and Lewis 1991; Manríquez-Morán et al. 2005), phylogeny (Censky and McCoy 1988), frequency of what are called nortes (i.e., storms that occurs from November to February on cool overcast days in the subtropics; Hernández-Gallegos et al. 2003), and time available for foraging and/or favorable conditions for oviposition (Vitt and Breitenbach 1993; López-Moreno et al. 2016).

For most species of whiptail lizards (genus *Aspidoscelis*), the selection of nest sites and the environmental conditions therein are poorly understood; however, we note the few known exceptions to this statement. Trauth (1983) searched for nesting habitat and oviposition sites of *A. sexlineatus* for 8 y, during which time he collected 195 egg clutches from 66 nesting sites in eight southeastern states in the USA. Nests of this species were typically excavated from the south to west sides of road embankments. Trauth (1987) also discovered nests of *A. gularis* by excavation at two sites in Texas, USA, at the base of south to west roadside embankments. In Jalisco state, México, Rodríguez-Canseco et al. (2013) reported eight neonates of *A. gularis* within a nest beneath a rock. The nesting data for *A. costatus* from Guerrero state, México, reported by Lara-Resendiz et al. (2013) is problematic as they presented no definitive evidence that the nests described there belonged to a single species. Contrary to the assessment of the authors of the presence of a single species, we have found at least three species of *Aspidoscelis* at their study site (Walker et al. 2020; unpubl. data).

Western Mexican Whiptail (*A. costatus*), a moderately large gonochoristic species endemic to western Mexico (Maslin and Secoy 1986), has been afforded special protection by Mexican laws (Secretaría de Medio Ambiente y Recursos Naturales 2019). Its Environmental Vulnerability Score (EVS) of 11 identifies it as a species of medium vulnerability (Wilson et al. 2013). According to International Union for the Conservation of Nature (IUCN) Red List, it is considered a species of Least Concern (IUCN 2007). In due course, *A. costatus* may be shown to be a complex of species. Currently, eight of its putative variants are recognized as subspecies (Reeder et al. 2002; Barley et al. 2019), one of which is *A. c. costatus* (see Maslin and Secoy 1986; Barley et al. 2019), commonly known as Balsas Basin Whiptail. In the inclusive sense, *A. c. costatus*

is widely distributed in the Mexican states of Guerrero, Mexico, Morelos, Puebla, Tlaxcala, and Oaxaca (Maslin and Secoy 1986; Méndez de la Cruz et al. 2018; Barley et al. 2019). It has an oviparous reproductive mode, producing a single clutch annually at our study site in central Mexico at 1,500–1,600 m elevation, which is the largest clutch size reported (mean of 7.7 eggs) within the genus *Aspidoscelis* (López-Moreno et al. 2016). The ovarian cycle is seasonal, spanning from the end of the dry season (April) to the middle of the wet season (July), with a higher prevalence of gravid females during June and July (López-Moreno et al. 2016).

Experience has shown that locations of nesting sites of female *A. c. costatus* (i.e., under volcanic rocks) in the high elevation study area are predictable, which facilitated completion of this study. Such predictability for nesting sites is not known for other Mexican population of *Aspidoscelis* at this elevation. Construction of the nest cavity beneath a volcanic rock for oviposition by a lizard allowed for the nests to be temporarily exposed by the researchers, fitted with sensitive data-recording devices, and restored to their natural condition. This allowed for assessment of thermal and hydric conditions for each nesting site, whether in or out of the nesting season of *A. c. costatus*, at a high elevation site in central Mexico. We evaluated these conditions in terms of their relationship to the reproductive cycle of female *A. c. costatus*.

MATERIALS AND METHODS

Nomenclature.—The nomenclatural status of Balsas Basin Whiptail (*Aspidoscelis c. costatus*), referred to as *Aspidoscelis c. costata* in publications following the erroneous recommendations by Reeder et al. (2002), was technically clarified by Tucker et al. (2016). Based on Article 30.1.4.2 (International Commission on Zoological Nomenclature 1999), the name *Aspidoscelis* must be treated as grammatically masculine rather than feminine as presumed by Reeder et al. (2002). This correction also impacted the suffixes of specific epithets of other Mexican whiptail lizards with names emended to end in -a, which should be returned to -us (e.g., *A. septemvittata* to *A. septemvittatus*). These revisions have been accepted by Barley et al. (2019), which included Tod W. Reeder among the coauthors.

Study site.—The study site was located in El Zapote, Municipality of Tonatico, Estado de México, México (18°45'17.1" N, 99°37'20.1" W; WGS84; elevation 1,500–1,600 m), which is an unusually high-elevation locale for teiid lizards of the genus *Aspidoscelis* (Vitt and Breitenbach 1993). The climate at the study site is semi-humid and warm with a rainy season occurring generally from June to September (Fig. 1). The rainy
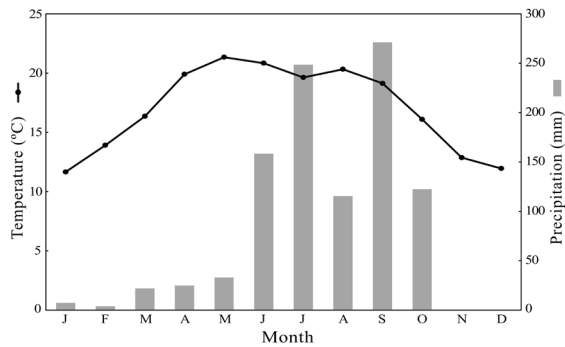
Figure 1. Average monthly air temperature (°C) and precipitation (mm) during the study period of the Balsas Basin Whiptail (*Aspidoscelis c. costatus*) in El Zapote, Municipality of Tonatico, Estado de México, México. (Data from Coatepequito Meteorological Station; http://clicom-mex.cicese.mx).

season may also include May and October given annual variations in some years (Hernández-Gallegos and Domínguez-Vega 2012). The patchy habitat consists of low-growing deciduous forest vegetation (Rzedowski 2006), interspersed among agricultural areas.

Field work.—From May 2008 to May 2009, we made weekly data collections during the nesting season of *A. c. costatus* (June-September), and conducted monthly field sampling during the remainder of the year. Based on preliminary findings, we searched for nests during each field trip in areas with elevations between 1,500 and 1,600 m, and with slopes of $11 \pm 2.4°$ (which can favor a greater retention of humidity, an important factor for the appropriate development of eggs; Flores-Santin 2010). We located all nests studied under volcanic rocks, with a mean length and width of 31.6 ± 1.4 cm and 14.0 ± 1.5 cm, respectively, and all rock coverings were completely exposed to the sun (Flores-Santin 2010). We ascertained that all nesting sites we located belonged to *A. c. costatus* based on the following: (1) some females were observed during actual oviposition; (2) the clutch size of 7.8 ± 0.58 eggs in the active nests (see below for the categorization) was not significantly different ($t = -0.54$, df = 29, $P = 0.590$) from the oviductal clutch size of 7.7 ± 0.44 eggs recorded by López-Moreno et al. (2016); and (3) both length (15.4 mm) and width (9.6 mm) of eggs were always smaller than the eggs of the Sack's Reticulated Whiptail, *A. sackii gigas* (18.6 mm in length, and 10.0 mm in width), the only other congener at the study site (Hernández-Gallegos et al. 2011). We recorded temperature and humidity for each nesting site whether in or out the nesting season. A thermocouple placed among the eggs under a rock recorded the temperature of each nesting site of *A. c. costatus* each hour (starting 8 May 2008) with a Hobo® Temp External Data Logger (Model H8, Onset Computer Corporation, Bourne, Massachusetts, USA). We evaluated the daily variation in temperature to understand proximate factors

relating to nesting and non-nesting behaviors using data from three nesting sites selected from April (warmest month, without active nests), August (a representative warm month, with active nests) and December (coldest month, without active nests). We measured the soil humidity at the nesting sites during each monthly field trip by a modification of the method proposed by McGee (1990). We took a soil sample of 50 g from beneath each rock and near each nest (i.e., within 5 cm from the nest perimeter), which we dried on a stove at 100° C for 24 h. Thereafter, the sample was weighed again to evaluate the loss of moisture. We recorded the moisture as a percentage according to the formula: percentage of humidity = (weight of the humid sample minus weight of the dry sample / weight of the dry sample) × 100 (Arzola-González 2007). Both temperature and soil moisture data, including the methodology, were taken in a very similar manner as Li et al. (2018).

Statistical analyses.—Because temperature and soil humidity did not meet the assumptions for parametric tests (Kolmogorov-Smirnov Test), and there were repeated measurements on the same nest over the months (i.e., non-independence in the data: autocorrelation), the differences among months were assessed using Generalized Linear Mixed Models (GLMM). This analysis also handled the cases of missing data due to loss and replacement of nests. We used the nest as the random effect, and the month as the fixed factor. We used Least Significant Difference to test for significant differences. Moreover, we estimated the thermal and hydric variances using the Coefficient of Variation (CV) per month, and we evaluated differences between CV of the nesting season and outside of the nesting season using the Mann-Whitney U test. We compared both temperature and humidity within nesting season in either active or inactive nests using either parametric or nonparametric tests. Additionally, we compared the temperatures of the nesting sites selected from April, August, and December using a Two-way ANOVA by hour, month, and the interaction of hour and month. We used SPSS version 20.0 for all statistical tests and we deemed results significant if $P \leq 0.05$.

Results

Number and types of nests found.—We identified two categories of nests for *A. c. costatus*: active nests (with live turgid white eggs or shell remnants that indicated hatching or fresh predation of eggs prior to their observation, Fig. 2A), and inactive nests (identified by brown eggshells from previous reproductive seasons, Fig. 2B). Accordingly, 26.7% were active nests (n = 12) found from June to September, whereas 73.3% (n = 33) were inactive nests found throughout the year. We
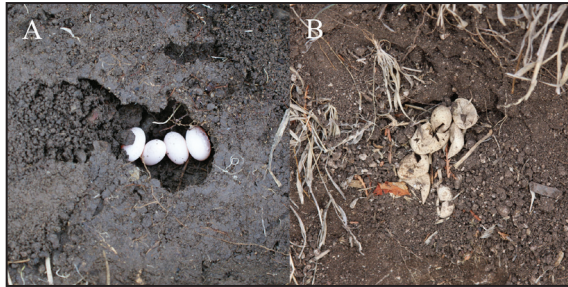
FIGURE 2. (A) Active nest and (B) inactive nest of the Balsas Basin Whiptail (*Aspidoscelis c. costatus*) in El Zapote, Municipality of Tonatico, Estado de México, México.

found no evidence of communal nesting. A chamber of mud was found in each active nest (Fig. 2). The eggs were not actually covered by soil, which likely enabled the hatchlings to leave the cavity more easily than if covered. We did not find a chamber in the inactive nests (Fig. 2). Moreover, the brown color of the eggshells was apparently caused by desiccation during the dry season. We base this on our observation that white eggshells we found in a nesting site in 2008 had become brown when we reexamined the nest in May 2009.

Variation in nest temperatures.—Temperature varied significantly throughout the months in the nesting sites ($F_{11, 34119} = 648.9$, $P < 0.001$). The nest effect was also significant ($Z = 5,693$, $P < 0.001$). During the nesting season from June to September (Fig. 3), the average temperature of all pooled data was 24.6° C (range 23.6°–25.6° C). During this season, both active (median 24.0° C) and inactive nests (23.6° C) significantly differed in temperature ($U = 39,315,210$, df = 8694, 9684, $P <$

0.050). Temperatures in the three months prior to the start of nesting (June) varied from 23° C to 26° C (March 23.9 ± 0.26° [standard error] C; April, 26.6 ± 0.26° C, the highest temperature of the year; and May 26.2 ± 0.28° C); however, no nesting sites including active nests were found during these months. We recorded the lowest temperatures in the nesting sites from October to February when there were no viable eggs; the mean temperature for these months was 19.2° C (range 17.4°–20.6° C). The nesting sites exhibited the greater thermal fluctuations outside of the nesting season ($U = 32.0$, df = 4, 8, $P < 0.050$; Fig. 4). The pattern of daily variation was similar in April, August, and December: the lowest temperatures within nesting sites were experienced in early morning, and the highest temperatures occurred in the afternoon (Fig. 5). Mean temperatures in the nesting sites varied significantly by the interaction of hour and month ($F_{46,144} = 2.810$, $P < 0.001$). Nest temperatures were hotter in April than in August, except between 0500 and 0900, when nest temperatures were either the same or hotter in August (Fig. 5).

Variation in nest soil humidity.—We found significant differences in the percentage of soil humidity throughout sampling in the nesting sites ($F_{10, 220} = 31.35$, $P < 0.001$, Fig. 3). The nest effect was nonsignificant ($Z = 1.209$, $P = 0.227$). During the nesting season (June-September), the percentage of humidity was the highest, with an average of 19.5% (range, 16.1–22.2%). During the nesting season, both active nests (19.7%) and inactive nests (19.8%) did not differ significantly in humidity ($t = -0.147$, df = 139, $P > 0.050$). Although we recorded suitable soil humidity for nesting in *A. c.*

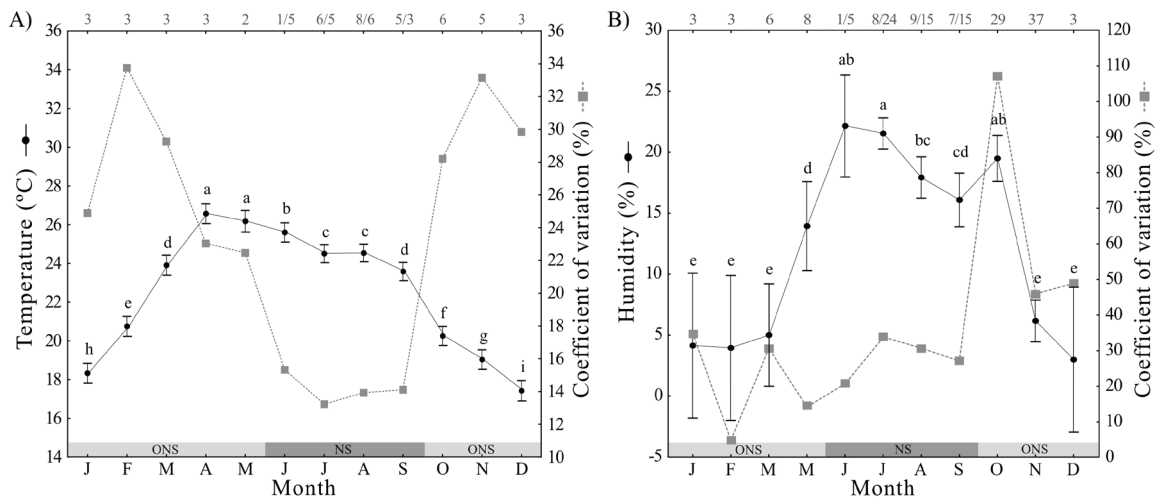**FIGURE 3**. Variation in (A) the average (95% confidence interval, solid line) of the temperature (°C) and (B) humidity (%) and their coefficients of variation (dashed line) per month measured in nests, within (NS) and outside of nesting season (ONS), of the Balsas Basin Whiptail (*Aspidoscelis c. costatus*) in El Zapote, Municipality of Tonatico, Estado de México, México. Different letters denote significant differences among months according to Least Significant Difference test ($P < 0.05$). Numbers at the top denote nests; single numbers are nests outside the nesting season, during the nesting season active nests are followed by inactive nests.
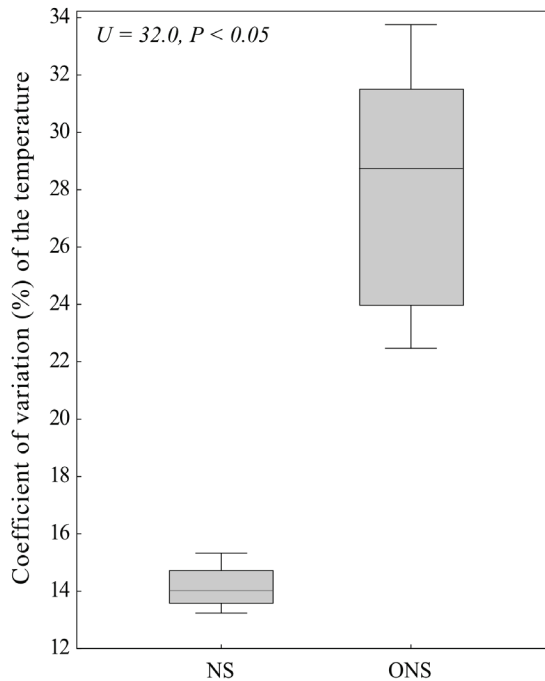
Figure 4. Comparison of coefficients of variation of the temperature in nests, within (NS) and outside of nesting season (ONS), of the Balsas Basin Whiptail (*Aspidoscelis c. costatus*) in El Zapote, Municipality of Tonatico, Estado de México, México.

Figure 5. Daily nest temperature (°C) variation during the hottest month (April, inactive nests), a representative warm month (August, active nests) and the coldest month (December, inactive nests) in nesting sites of the Balsas Basin Whiptail (*Aspidoscelis c. costatus*) in El Zapote, Municipality of Tonatico, Estado de México, México.

costatus in October (19.5 ± 0.96%), active nests were not found in that month. From November to March the lowest yearly percentage in humidity was recorded with an average of 4.5% (range, 3–6.2%). The CV for the humidity demonstrated that during the nesting season, the variation did not differ significantly from other months ($U = 20.0$, df = 4, 7, $P = 0.298$; Fig. 3).

Discussion

Incubating reptile eggs are subjected to fluctuating environmental factors, of which temperature and soil humidity are among the most important (Calderón-Espinosa et al. 2018; Li et al. 2018). According to Brown and Shine (2006), Li et al. (2018), and the results from our study, female *A. c. costatus* selected a moist and warm environment with minimal temperature fluctuations under rocks (between June and September). This condition is favorable for oviposition and embryonic development and provides suitable micro-environmental conditions to stimulate oviposition progressing to successful incubation. The nest effect was also significant, which could have resulted from the sizes of the covering rocks.

Both daily and monthly temperature fluctuations in nests seem to be a common pattern in lizards (Qualls and Shine 1998; Calderón-Espinosa et al. 2018; this study). This suggests that oviparous lizards have
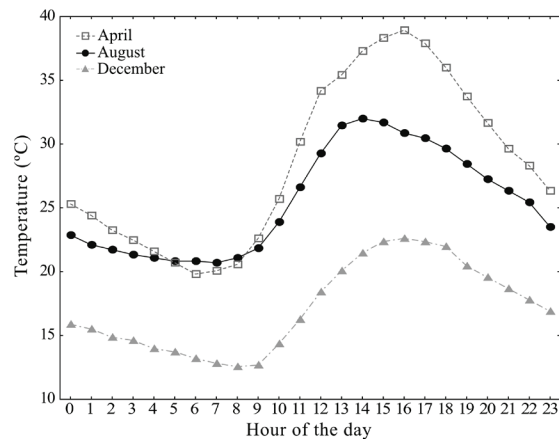
evolved physiological mechanisms that withstand such temperature fluctuations (within specific ranges) with minimal disruptions to the developmental processes, whereas prolonged egg retention (atypical in *Aspidoscelis*) would both reduce such fluctuations and preclude evolution of such mechanisms. The nesting environment that the eggs of *A. c. costatus* naturally experience indicated that the average incubation temperature was 24.6° C. The incubation temperature was similar to those recorded for other whiptail lizard species such as Chihuahuan Spotted Whiptail, *A. exsanguis* (22°–26° C), Six-lined Racerunner, *A. sexlineatus* (25°–32° C), and Sonoran Spotted Whiptail, *A. sonorae* (22°–26° C; Köhler 2005); and is found within the optimal temperature for oviparous reptiles in general (23°–31° C; Birchard 2004). In *A. c. costatus*, incubation temperature possibly influences the embryonic developmental process (Andrews et al. 2000; Parker and Andrews 2007; López-Moreno 2011), and the different phenotypic traits of the hatchlings (Andrewartha et al. 2010; Esquerré et al. 2014). At El Zapote, Municipality of Tonatico, which is a high-elevation (1,500 and 1,600 m) population of whiptail lizards (Vitt and Breitenbach 1993), the rocks permanently exposed to the sun heated up to 47.4° C; however, the temperatures of the nests underneath did not exceed 37° C. The protective effect of the rock cover for temperature extremes was noted by Flores-Santin (2010) for high temperatures and by Calderón-Espinosa et al. (2018) for lower temperatures. We regard this as an adaptive response for populations at high elevations (Shine 2004; Calderón-Espinosa et al. 2018; Granados-González et al. 2020) including the El Zapote site. This response provides eggs of female *A. c. costatus* a thermal micro-environment with relatively

minimal fluctuation for incubation (i.e., the rocks reduce both absolute maximum and minimum values of temperature). In our population, a very similar situation was recorded for the nesting sites during 2009 and 2010 (López-Moreno 2011). Increased thermal variation during incubation has been found to significantly reduce embryonic survivorship and offspring fitness (Horne et al. 2014; Li et al. 2018).

Our study shows that moisture from rainfall influences nesting microhabitat; the egg-laying season in *A. c. costatus* occurs during the months with more rainfall and represents an adaptation responsive to these conditions. Based on results for other species (Packard and Packard 1987; Vleck 1988), this adaptation helps female lizards to avoid habitats and/or time periods that are prone to desiccating eggs (Li et al. 2018). We believe that eggs incubated under adequate humidity conditions, such as a mean of 19.5% for our population of *A. c. costatus*, would produce larger individuals in June to September. This would not occur under drought conditions, such as those recorded under rocks in El Zapote from November to May, where the albumin in the eggs could not be separated and degraded (Packard et al. 1977).

Seasonal reproduction in squamates has been hypothesized to be correlated with both biotic (i.e., evolutionary baggage, resource availability) and abiotic factors (i.e., temperature, rainfall, frequency of nortes, day length). This included appropriate micro-environmental conditions (i.e., thermal and hydric conditions) for oviposition/incubation (Brown and Shine 2006; López-Moreno et al. 2016). Our data further supports the hypothesis by López Moreno et al. (2016) that the female reproductive cycle of *A. c. costatus* is likely correlated with the thermal and hydric conditions at the nesting sites. At El Zapote the required temperature and soil humidity for the embryonic development of *A. c. costatus* only occur simultaneously in June through September. Any incubation scenario beyond the optimal values presented are thought to be inadequate for the embryonic development of *A. c. costatus*; no active nests have been found before or after this period. It should be noted that there are months which are within, or close to, the interval for either temperature or humidity, but not for both simultaneously. From March through May, an adequate incubation temperature was present, yet the soil humidity was lower than the average values. During October, the soil humidity was adequate, yet the temperature was also lower than the average values observed in the nesting season. The oviposition timing is significant for the success of egg hatching (Warner and Shine 2007). If the eggs of *A. c. costatus* were oviposited before June, viable embryos would likely not develop because of inadequate humidity for yolk metabolism (Packard et al. 1977; however, further experimental evidence is needed

to corroborate this hypothesis). If the oviposition took place in October, the eggs would be exposed to proper humidity but not an adequate temperature and similarly there would be no success in the incubation, as has been demonstrated by López-Moreno (2011).

Investigations into other sympatric oviparous species (i.e., *A. sackii gigas*) are needed to compare the warm (including low variation in temperature) and moist environment inside of nests during nesting season that was observed in *A. c. costatus* is consistent across teiid lizards. This topic would provide a greater understanding of the nesting ecology of lizards and its relationship with different biological traits including female reproductive cycle. Although *A. c. costatus* is still widely distributed in south-central Mexico, it can be locally threatened by a variety of anthropogenic activities such as habitat loss and land conversion (Gómez-Benitez et al. 2016). In addition to destruction of local populations (including decline and collapse), recent studies indicate that these anthropogenic activities apparently promote potentially harmful morphological and ecological changes in this species (Gómez-Benitez et al. 2016, 2020; Gómez-Benitez 2017). Successful, evidence-based conservation of vulnerable populations of *A. c. costatus* requires robust basic natural-history data (i.e., nesting environment), which will provide knowledge of the effects of both anthropogenic and natural factors in its life history. This is especially needed in areas where preferred habitat loss of *A. c. costatus* is the primary threat to a lizard population as reported for *A. gularis* in Mexico City by Hernández-Gallegos et al. (2009).

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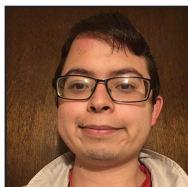
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