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Reproduction in the green alligator lizard *Abronia graminea* (Squamata: Anguidae) Cope 1864.

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ABSTRACT:

Abronia graminea is a viviparous species that inhabits pine-oak and cloud forests in the highlands of the Mexican states of Puebla, Oaxaca and Veracruz. Parturition occurs during the late dry season or early rainy season (March through May). We describe its sexual morphological characteristics and record the annual reproductive. Examining a group of 114 Abronia graminea, all specimens were sexed by hemipenies evagination technique. Sexual dichromatism was used in a morphometric analysis of this population, including determining length differences between sexes, by comparing head length, head width, tail length, tail base width, and snout-vent length. We assessed the sexual cycle by observing mating activity and parturition. We determined the sex of 55 males, 53 females, and 6 juveniles. Different on color patterns were observed between sexes. Males are uniform bright green, while females are duller green with spots or bands. Using a "T" tests for each comparison we observed significant differences between head length, head width, and tail base width of adult males and females. We did not find significant differences between adult male and female tail lengths and snout-vent lengths. The sexual cycle of A. graminea was described for captive mating activity and parturition in this population. Mating activity was observed in October. Parturition of young from a total of 49 litters was recorded. Newborns are brownish color with dark transverse dorsal bands.

Key words: Reproduction, Captive breeding, Reproductive cycle, Sexual dimorphism, Dichromatism.

RESUMEN

Abronia graminea es una especie de lagartija vivípara que habita bosques de pino-encino en las zonas altas de los Estados mexicanos de Puebla, Oaxaca y Veracruz. Los partos ocurren durante la temporada de secas o a principios de la temporada de lluvia (entre Marzo y Mayo). El artículo describe las características morfológicas sexuales y registramos el ciclo reproductivo anual. Se examinó un grupo de 114 individuos de Abronia graminea. Todos los especímenes fueron sexados por evaginación de hemipenes. Se usó el dicromatismo para hacer un análisis morfométrico de ésta población, que incluyó la determinación de diferencias en tamaño entre sexos, se compararon los largos y anchos de las cabezas, largo de la cola, ancho de la base de la cola, y longitud hocico-cloaca. Se evaluó el ciclo sexual observando las copulas y los nacimientos. Se determinaron 55 machos, 53 hembras y 6 ejemplares juveniles. Se observaron diferencias en patrones de coloración entre los sexos. Los Machos fueron color verde brillante uniforme, mientras que las hembras fueron color verde más pálido con manchas o bandas. Usando un análisis de "T" de Student para cada comparación se observaron diferencias significativas entre los largos y anchos de las cabezas, así como entre los anchos de las bases de las colas, entre machos y hembras, y no se encontraron diferencias significativas entre los largos de las colas y las longitudes hocicocloaca. El ciclo sexual de A. graminea fue descrito por la actividad de cópulas y nacimientos en cautiverio de esta población. La actividad de cópulas fue observada en Octubre. Se registraron 49 nacimientos. Las crías presentaron un color pardo con bandas dorsales transversales.

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Palabras clave: Reproducción, Reproducción en cautiverio, Ciclo reproductivo, Dimorfismo sexual, Dicromatismo.

INTRODUCTION

Abronia graminea inhabits pine-oak and cloud forests with large quantities of epiphytes (Campbell and Frost, 1993) in the highlands of the Mexican states of Puebla, Oaxaca and Veracruz, (Tihen 1949, Smith and Taylor 1950, Good 1988 in Schmidt 1991), where the average temperature is 16.1 C°, the highest temperatures occur in April and May, and the lowest in December and January. The rainy season extends from June to September, and the mean elevation is 1,782.9 m above sea level. (www.inegi.com.mx). Adults usually reach about 106 mm snout-vent length (SVL; Campbell and Frost 1993).

Reproduction has been studied in many lizard species; lizards exhibit a great variety of reproductive strategies, including oviparity and viviparity. Viviparous species are commonly found in high elevations; viviparity provides protection against predators and aids in thermoregulatory adaptation (Guillette 1980). Abronia graminea is a viviparous species similar to most species of Anguids (Werler 1951). The live-bearing habits are highly advantageous to species in cooler climates at high latitudes and at high elevations such as the pine-oak and cloud forests inhabited by A. graminea (Greer 1967). Werler in 1951 reported a female giving birth to 4 young on April 12. Campbell and Frost (1993) and Schmidt (1991) noted that copulation of this species occurs in the leaf litter on the ground during early August. The male holds the female with his body and grabs her head firmly with his jaws. This activity can last up to 12 hours. Birth occurs during the rainy season, there are some records of females from Aculzingo that gave birth in July and August (Cardon pers. comm.). Newborn length is about 30 mm (SVL). They are copper colored, banded with dark stripes, and therefore strikingly different from the adults. There are 1–12 newborn, the number depending somewhat on the age and size of the female (Alvarez del Toro 1992; Campbell and Frost 1993).

Abronia graminea is listed by IUCN as endangered (www.iucnredlist.org), because their populations are fragmented; its habitat has been diminished by deforestation, natural fires, and human interference. They are subject to illegal collection for the pet trade, and are often killed because they are erroneously considered to be venomous.

There is little published information on captive breeding and husbandry of this species. Available published information consists mostly of anecdotal observations. Reproduction is one of the most important aspects of any captive conservation project. Information about courtship, copulation, and gestation or incubation is critical to development of the good husbandry techniques required to correctly manage captive populations (The International Training Center for Breeding and Conservation of Endangered Species 1995). In some cases the sexual differences between genders are not obvious, and it is therefore important to analyze the morphological characteristics of the species to distinguish gender. Another crucial aspect is to describe key events in the reproductive cycle, such as mating, egg laying and hatching, or birth.

Our goals were to describe external sexual morphological characteristics and record the annual reproductive cycle of *A. graminea*.

MATERIAL AND METHODS:

Our primary database consisted of 114 individuals of *Abronia graminea* confiscated by the Mexican authorities and turned over to us. The animals were collected by "local collectors" presumably from the highlands around Xalapa and Aculzingo in the state of Veracruz, and destined for sale in the illegal trade.

The lizards were maintained in four different types of enclosures. Four males were kept in a 62 x 47 x 66cm glass terrarium. The remaining males were kept in groups of 3 individuals within 27 x 52 x 30 cm glass tanks, each of which had a plastic lid attached with suction cups. Six individual females were housed in a 66 x 47 x 45cm glass terrarium. The remaining females were placed in terraria measuring 31 x 27 x 18cm and 33 x 39 x 27 cm, 31



x 27 x 18 "Rubbermaid" multiuse plastic boxes and 33 x 39 x 27 "Kip" plastic multiuse boxes. Juveniles were placed in groups of 1–3 within 31 x 27 x 18cm plastic multiuse boxes. All had mosquito mesh fixed on the lid for ventilation. All terraria were furnished with sphagnum moss as a substrate and included branches and live plants, for climbing. Terraria were placed facing a window in a sunny room. The light cycle and temperature were similar to their natural habitat.

All animals were sexed by hemipenial eversion. Sexual dichromatism assessment and a morphometric analysis were also done in order to determine differences between sexes. To determine the significance of secondary sexual characters of the animals, we did a Student's T test analysis (Bruning and Kintz, 1977) with a level of confidence of 95% for each comparison of each feature between sexes. Significant differences between head length head width, tail length, tail base width, and snout-vent were noted. We also described the sexual cycle by observing mating activity and parturition.

We assessed measured sexual characteristics that differentiate males from females. Morphometric measurements were taken using calipers (± 0.05 mm) of the following features (Table 1) were taken using calipers (± 0.05 mm):

head length (HL) head width (HW) tail length (TL);

- a) tail base width (TBW) and
- b) snout-vent length (SVL).

We analyzed these data using different T' tests for

each comparison to determine if there were significant differences between the total lengths of adult males and females in this population:

RESULTS

We determined the sex of 114 individuals, including 55 males, 53 females, and 6 juveniles. Different colorations were observed between sexes. Adult males had a uniformly bright emerald green dorsum (Fig.1a), whereas females were duller green, exhibiting spots or bands on the dorsum that were sometimes brownish, yellowish, or whitish (Fig. 1b). We observed uniformly emerald green and brown-banded juveniles; the former were males (Fig. 2a) and the latter females (Fig. 2b). We corroborated these observations by dissecting 5 juveniles.

Morphometrics and sexual dimorphism:

Head Length

We observed significant differences between head lengths of adult males and females (Fig. 3) based on analysis of a sample of 15 adult males and 15 adult females. Mean head length for males was: 29.6 mm and for females: 26.3 mm.

Head width

We observed significant differences between adult male and female head width, based on a sample of 15 adult males and 15 adult females (Fig. 4). The mean head width of males was 21.53 mm and 18.27 mm for females.

 $Table \ 1. \ Morphometrics \ between \ adult \ males \ and \ females, \ SVL=media \ snout-vent \ length, \ TL=media \ tail \ length, \ TBW=media \ tail \ base \ width, \ HW=media \ head \ width, \ HL=media \ head \ length.$

Character	N males	N Females	mean males	mean females	T	P	N total	DF	SD males	SD females	F variances	P variances
HL	15	15	29.6	26.30	7.79	0	30	28	1.12	1.22	1.89	0.75
HW	15	15	21.53	18.27	8.55	0	30	28	1.41	0.46	9.45	0
TL	36	27	150.42	148.44	0.34	0.69	63	61	0.25	20.74	1.59	0.22
TBW	15	15	15.07	8.80	1.17	0.25	30	28	0.52	0.56	1368.85	0
SVL	15	15	110.67	110.67	0	1	30	28	5.77	4.58	1.59	0.4

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Fig. 1. Sexual dichromatism (a) male, uniformly bright emerald green, (b) female, greenish, with brown and whitish markings.



Fig. 2. Sexual dichromatism in juveniles (a) male, solid green; (b) female green banded with grayish patches.

Tail length

Only individuals with unregenerate tails were used in this analysis. We did not find significant differences in relative tail lengths between males and females (Fig. 5) using the T test to compare tails in 36 adult males and 27 females. Mean tail length for males = 150.42 mm and mean tail length for females = 148.44 mm.

Tail Base Width

We observed significant differences between adult male and female tail base widths, based on 15 adult males and 15 adult females (Fig. 6), using T test. The mean width for males = 15.067 mm and for females = 8.800 mm.

Snout-Vent Length

We did not observe significant differences in snoutvent length in 15 adult males and 15 females



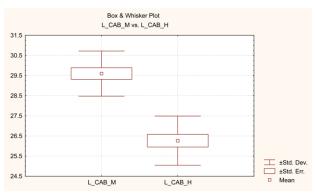


Fig. 3. Head length (HL).

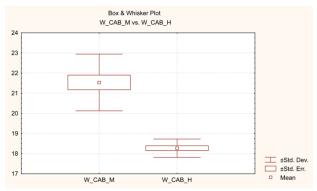


Fig. 4. Head width (HW).

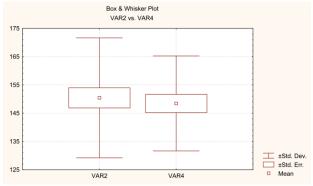


Fig. 5. Tail length (TL).

(Fig. 7). The mean SVL for both sexes = 110.667 mm.

Sexual cycle

The sexual cycle was deduced by following mating activity and parturition in this population. We recorded mating activity through written

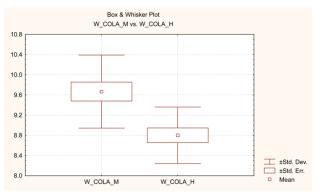


Fig. 6. Tail base width (TBW).

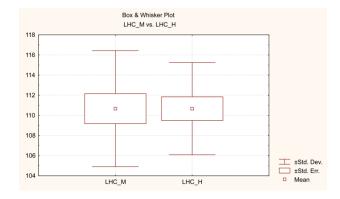


Fig. 7. Snout-vent length (SVL)

descriptions, photographs and videos. Two instances of mating were observed during this study, one on 1 Oct 2000, in which male Number 44 copulated with female Number 49, which was videotaped (Fig. 8), and one on 2 Oct 2000, in which male Number 28 and female Number 26 copulated for more than 12 hours.

Copulation

During copulation, the male pursues the female, grasps her by the temporal region of the head, and drags her wherever he goes, while the female remains motionless. Male introduced one hemipenis into the female's cloaca. The male's tail often undulates during copulation. The total duration of mating was more than 12 hours. After these two mating records males and females were separated.





Fig. 3. (a) Mating activity between male No. 44 and female No. 49 on 1 Oct 2000. (b) Mating activity between male No. 28 and female No. 26 on 2 October 2000.



Fig. 4. Aggressive display between newborns Abronia graminea

Parturition

Several of the females arrived gravid and gave birth. These animals were collected illegally and the knowledge or facilities were not available to provide adequate care. Therefore, no data were recorded on either females or their litters and the majority of the newborn died. Survivors were managed separately from adults afterwards.

Parturition of 49 litters was recorded between 22 Mar and 13 May 2000. There was a total of 193 young; of these 18 were born dead and 1 was deformed. The smallest litter recorded contained only one young, the largest contained 12 (mean = 4). Most of the females gave birth, (92%, n = 53), providing evidence that females reproduce yearly. Gestation lasts from 5 to 10 months, assuming that fertilization occurred in early August as was reported by Campbell and Frost (1993) and Schmidt (1991) or in October as we observed during this study based upon and birth was observed between March and May. But we are aware that these captive conditions were not the adequate ones, and because they were coming from illegal trade these animals were exposed to high levels of stress, which could affect the normal breeding cycle.

Gravid females may have been collected gravid intentionally to establish a reproduction colony by commercial breeders. During the spring of 2001 and 2002 no births were recorded; therefore spermatogenesis and/or vitellogenesis may require special environmental stimuli or husbandry

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provided to the animals in captivity was somehow inappropriate.

Young

Newborn are brownish with dark transverse bands: they are very different in coloration from the adults that are green. Newborn are tame and delicate in captivity; the majority of these individuals died within the two years of this study. Newborn are mainly terrestrial; none of them were observed climbing, even though this genus is characterized as arboreal. Newborn remained mainly hidden beneath the substrate, in this case leaf litter, in which there were camouflaged. The mean SVLat birth was 32 mm and at one year was 48.1 mm.

Newborn did not eat during the first days of life. After some days they showed interest in food, but they had difficulty striking the prey accurately and missed most of the time. The food items that most attracted juveniles were soft-bodied insects such as wax worms. We also observed that they were more inclined to feed when there were multiple individuals in the terrarium. Juveniles exhibited aggression toward other juveniles, often biting each other (Fig. 9). Several newborns never showed any interest in food and died within a few days after birth. At one year of age individuals started losing their brownish color, changing green; several of them had dark transversal bands..

DISCUSSION

Sexual dimorphism

In captive management programs it is crucial to recognize characteristics of males and females within the captive population. Some lizards show sexual dimorphism (e.g., male iguanids and phrynosomatids have more developed femoral pores than females. Anguids lack such pores, but some species like *Barisia rudicolis* (Zaldivar-Riveron 1998) exhibit different adult colorations (e.g., sexual dichromatism). We found that *A. graminea* exhibits sexual dichromatism, with males being uniformly bright green and females being duller green and sometimes are spotted or banded with brown, gray, yellow or white (Campbell and Frost 1993). In other species of reptiles males are also

bright green and females are more mimetic to the substrate, so that predation may be higher on males than on females, since gravid females are more valuable than males for the population (Halliday 1980), Even though, males could be more difficult to visualize within dense foliage. We observed sexual dichromatism in juveniles at as early as six months. These differences of coloration in young *A. graminea* have also been observed in other captive situations (Cardon *pers comm.*).

Morphometrics

In this study we searched for other sexual characteristics by taking measurements of bodies, heads and tails of all individuals of this population. We found no differences between SVL and tail length of males and females, similar to another anguid, *B. rudicolis*(Zaldivar-Riverón 1998). We found differences between males and females for tail-base width, head length, and head width. The genus *Barisia* also shows sexual dimorphism for head width (Vial and Stewart 1989; Zaldivas-Riverón 1998). The head length and head width is associated with copulation. During mating males grasp the heads of females so larger heads presumably result in more successful mating (Vial and Stewart 1989).

Sexual cycle

Abronia graminea is a viviparous species with ovulation occurring in fall, gestation in winter, and parturition during spring and summer, similar to other viviparous lizards from high elevations in tropical areas (Méndez de la Cruz et al. 1998). Abronia graminea appears to be sensitive to specific changes in the environment. For example, poor or total lack of UV light may reduce or eliminate sexual behavior, as was observed during fall 2000, copulation was not observed. subsequently supplemented all terraria with UV light as recommended by De Graw (per comm.) and Hudson (per comm.), and sexual behavior was elicited. These animals gave birth during April corresponding an early birth for this species this may be due its origin from illegal traffic, then maybe females were under strong stress due to improper transportation and inadequate husbandry



techniques that force this females to give birth earlier than normal, this early births have been reported for *Crotalus polystictus* in captivity by Cardon (*per comm.*), leading to a improper seasonal cycle, in the middle of a dry season, reducing the survival of the new births.

Mating

The copulatory behavior of males involves pursuing females and grasping their heads in their jaws. This behavior is consistent with what has been reported for other species of lizards, such as *Ctenophorus maculosus*. Males of this species may bite the female's head with such force that it sometimes kills the female (Olsson 1995). We did not observe such aggressiveness in *A. graminea*. Copulation was observed during October as was previously reported by Campbell and Frost (1993) and by Schmidt (1991). Each event lasted for more than 12 hours consistent with information reported by Schmidt (1991), but contrary to what was reported by Campbell and Frost (1993), who reported copulas of just 45 minutes.

Parturition

During this study we recorded 193 newborns from 49 litters, from 22 March to 13 May 2000. These data are in agreement with the report by Martin del Campo (1939), when he observed an A. taeniata individual giving birth to 4 young on 12 April. Graham (2001) reported the birth of 4 A. graminea on 21 March 2001; Cardon (per comm.) also observed several births in Mar 2002. During this study we observed litters of 1–12 offspring, similar to Campbell and Frost (1993), and the median litter size was fourth. Of the total of 193 offspring, 18 were born dead and one was deformed. During 2001 and 2002 no births were observed; we believe this was caused by stress and inadequate captive conditions (Mendez de la Cruz et al. 1998).

Newborn and juveniles

Newborn are conspicuously different from adults, similar to other gerrhonotine species. For example, Zaldivar-Riverón (1998) reported

newborn of *B. rudicolis* are brownish grayish with seven or eigth transversal bands, whereas adults are olive green or yellowish. Campbell and Frost (1993), and Graham (2001) mentioned that newborns of the genus *Abronia* are usually copper color with transversal bands, and we observed this during our study. The mean SVL for newborns was 32 mm, slightly smaller than what Campbell and Frost (1993) reported for *A. mixteca*.

CONCLUSIONS

- 1. We describe the sexual dichromatism for *A. graminea* in both adults and juveniles.
- 2. Significant differences are described between adult males and females for head length and head width, as well as in the tail base width, but we did not find significant differences between adult male and female SVLength or in tail length.
- 3. *Abronia graminea* has a sexual cycle involving a fall ovulation and winter gestation.
- 4. The sexual activity of this population was concordant what was described by other authors for this species.
- 5. We observed 193 births and provide details on these
- 6. The parturitions happened during April (dry season), this may be caused by stress of inadequate transportation and poor husbandry techniques.

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