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Reproductive biology in the wild and in captivity of *Anolis aquaticus* (Sauria: Polychrotidae) in Costa Rica

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ABSTRACT

The reproduction of *Anolis aquaticus* (Taylor 1956) of La Palma de Puriscal creek, Costa Rica (9°45'N, 84°27'E) was studied. Marking and recapturing methods were used in the field during 48 study days over a 16-month period. Females reached their sexual maturity at an earlier age and less snout to vent length (SVL) than males, and were found to have slower growth rates. Males were recruited in greater numbers and had higher survival rate than females. During mating, the female chose the site of copulation. Copulation lasted between 90 and 105 seconds; no post-copulatory courtship was observed. Complete mating showed: The mating site chosen by the female, a male approaching to the female, courtship with gular sac display, male body swinging, female body inspection executed by the male including body swinging, tongue´s tip caressing by the male on the hip of the female, mating, copulation, and separation of the couple. The complete copulating process last between 90-105 seconds; there was no post-copulation courtship. Reproduction occurred throughout the year with one or two eggs being laid per clutch. *Anolis* deposits their eggs in crevasses with intervals between clutches of 20 to 40 days. In captivity, the average incubation temperatures were between 22°C and 25°C, and the incubation time was between 75 and 82 days. Under natural conditions the incubation temperatures were found to be between 19°C and 23°C. Males were found to have slightly greater growth rates and to reach greater body sizes at the same age.

Key words: *Anolis aquaticus* reproduction, Costa Rica

RESUMEN

Se estudió la reproducción de *Anolis aquaticus* Taylor 1956 de la quebrada La Palma de Puriscal, Costa Rica (9º45'N, 84º27'O). Métodos de marcación, recaptura, jaulas para desoves, incubación y monitoreo de temperaturas fueron utilizados en el campo durante 48 días en 16 meses. Las hembras

alcanzaron la madurez sexual a menor edad y a menor tamaño en longitud hocico-ano que los machos, y presentaron tasas de crecimiento inferior. Los machos fueron reclutados en números más altos, y sobrevivieron más que las hembras. Dos cópulas completas y una incompleta fueron registradas. Cópulas completas muestran escogencia del sitio por la hembra, acercamiento del macho a la hembra, cortejo con despliegue de abanico gular, balanceo del cuerpo del macho, inspección del macho a la hembra incluyendo balanceo del cuerpo, pasarle la punta de la lengua en la cadera de la hembra, monta, copulación y separación de la pareja. Las cópulas completas tardaron entre 90 a 105 segundos; no existió cortejo post-copulatorio. Se reproducen todo el año y desovan de uno a dos huevos por camada. *Anolis* depositan los huevos en el interior de fisuras, el tiempo entre un desove y otro fue de 20 a 40 días. En cautiverio, las temperaturas promedios de incubación fueron de 22º a 25ºC, y el tiempo de incubación fue de 75 a 82 días en cautiverio, y de 19º a 23ºC en condiciones naturales. Los machos presentan tasas de crecimiento ligeramente superior a la de las hembras, y alcanzan mayor tamaño corporal a la misma edad.

Key word.- Reproducción, *Anolis acuaticus*, Costa Rica.

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INTRODUCTION

The species of the *Anolis* genus (Sauria: Polychrotidae), commonly known as anole, are distributed from the United Stations to Brazil and the Caribbean, from sea level to altitudes of 2000 m above sea level, depending on the species (Williams, 1976; Duellman, 1987; Guyer and Savage, 1986; Frost and Etheridge, 1989; Savage and Guyer, 1989; Rodriguez and Larramendi, 2003; Márquez *et al.,* 2005). Lizards of the genus *Norops* are among the smallest and most numerous of the infraorder Iguania, and occupy a wide variety of microhabitats throughout the neotropics, from the arboreal to the semiaquatic. Few species have been studied in detail (Campbell, 1973; Hertz, 1975; Rand & Rand, 1976).

There are 21 species of the *Norops* genus in Costa Rica and five of the *Anolis* genus (Taylor, 1956; Fitch, 1970; Savage & Villa, 1986). The *Norops* and *Anolis* have diurnal habits and eat invertebrates that they capture amongst fallen leaves and shrubs during the day. The prey of the adults of *N. limifrons* (Cope, 1862) *N. polylepis* and *A. garmani* (Stejneger, 1899) have an average length of 8-9 mm (Andrews, 1971; Sexton *et al.,* 1972; Campbell, 1973; Andrews and Rand, 1990). The body length of the *Norops* of Costa Rica varies from 41 mm (*N. humilis* Peters 1863) to 160 mm (*A. insignis* Cope, 1871) (Savage & Villa, 1986).

Of the 21 species of *Norops* of Costa Rica analysed by Taylor (1956), the population of this study is considered to be a patch of the species *A. aquaticus*. Consequently, I refer to this species as *A. aquaticus*. During territorial displays in conflicts between males and in courting females, the males repeatedly

extend the dewlap that they have under their neck, and jerk the head and body up and down, according to the typical pattern of the species to which they belong (Andrews & Asato, 1977). Some species of *Anolis* in the Caribbean extend the dewlap and attempt to bite the adversary to defend themselves (Rodríguez & Larramendi, 2003). The same authors note that *A. vermiculatus* and *A. bartschis* do not have dewlaps and in their place have a small transverse fan that does not extend like those of other species. The females of *A. isolepis* of Cuba have also a dewlap of almost the same dimensions of that of the males. Crews (1973) observed that severing the hioideo cartilage of captive *A. carolinensis* males left them unable to extend the dewlap to court females and the females did not respond sexually to the courting displays of the males. Andrews (1971) states that uses of dewlap by males are to advertise their presence in front of both females and males and to court females.

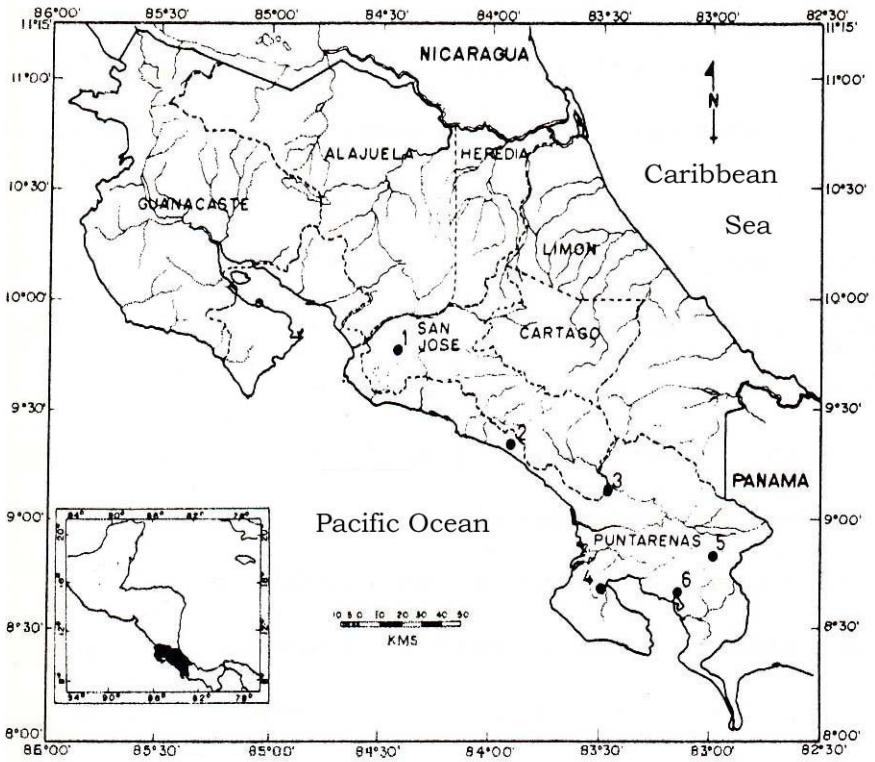

Figure 1. Location of the study site and distribution of *Anolis aquaticus* in Costa Rica.

There are no detailed studies concerning the reproductive behavior of *A. aquaticus* (Taylor 1956) in Costa Rica. In this study the reproduction of the species is investigated, including examination of dewlap display as a form of sexual attraction for mating and courting, mounting, copulation, nesting sites, size, egg colour, incubation temperatures in natural and semi-natural conditions, hatching, size of hatchlings, and growth rates in the wild and in captivity.

METHODOLOGY

Study area.- La Palma de Puriscal creek is located at 16 km from Santiago de Puriscal on the old route to Quepos. It belongs to the province of San José, Costa Rica (9°45' 00"N, 84°27'00"E) at 910 m a.s.l. It begins in the foothills of the Turrubares in the northern sector of the Talamanca mountain range and leads to the Quibel River. In the dry season the average channel width is 0.89 ± 0.30 m (0.50 - 1.44 m, n = 15 measurements), while in the rainy season the average channel width measures 1.09 ± 0.34 m (0.50 - 1.64, n = 15 measurements in the same locations). Along the creek there is a riparian forest, shrubs and grassland that begin at the water's edge and extend to 50 - 100 m outside the creek, succeeding to pastures on either side. The study site was classified, according to the zonal system of Holdridge, in montane rain forest (Valerio, 1991). According to the types of vegetation of Costa Rica (Gómez, 1986), the area is described as seasonal tropical rain forest of medium altitude. In terms of climate, the area is wet with a moderate rainy season and a short dry season (Herrera, 1985; Fig. 1).

Field methods.- The investigation was carried out from the end of March, 1991 to March, 1993. Each animal was manually captured and marked by the cutting of claws. To mark the animals, the methods of Tinkle (1967) and Medica *et al.* (1971) were used as references. In order to mark the animals by cutting a minimum number of nails, the two methods were modified in the following manner: to the toes of the front right foot the numbers 1, 3, 5, 7 and 9 were assigned; to those of the front left foot the numbers 2, 4, 6, 8 and 10 were assigned; to those of the back right foot the numbers 30, 50, 70, 90 and 200 were assigned; and to those of the back left foot the numbers 20, 40, 60, 80 and 100 were assigned., One or two nails were cut with scissors on each foot of the animals and the corresponding number noted. The SVL was measured with calipers from the mentoniane scale to the superior lip of the cloacae; the length of the tail was also measured from the inferior lip of the cloacae to the extreme tail tip. To measure the weight, the animal was tied with fine dental floss and suspended from 10 - 50 g scale. the animals were subsequently released.

Sexual maturity of males and females.- To determine the sexual maturity of males and females, 11 females and 12 males were dissected, measuring between 37.45 and 77 mm in SVL. The gonads were observed and measured with a Reichert Zoom microscope. The recapture data (3 - 7 times for females, and 3 - 8 times for males) and growth rates of animals less than 32 mm in SVL were used to estimate approximate age, and the age at which males and females reach sexual maturity. According to the size of the juvenile lizard had at first capture its age was estimated in between 30 to 45 days old (with reference to the size of the two hatchlings obtained in captivity which were 24 - 25 mm at hatching). Sexual maturity was reached in females that exceeded 52 mm in SVL and in males that exceeded 55 mm in SVL. Calculations were carried out to compare the final and initial sizes, dividing by the number of

months elapsed which gave values of 4 - 6 months for females to reach sexual maturity and 5 - 7 months of males to reach sexual maturity.

Courting and copulation.- Three courting couples were observed in natural conditions from distances of 3 - 5 m. All of the movements made by each of the participants during courting, copulation and after copulation were noted.

Oviposition and incubation.- Females greater than 53 mm in SVL were examined by touch to establish if they were gravid in the following manner. The female was placed in a ventral position and the index finger and thumb were used to gently touch the ventro-inguinal region for 6 - 10 seconds. If one or two oval protrusions were felt, these were considered to be eggs. This was later confirmed by the dissection of several females.

The 11 females in captivity laid six eggs. These six, plus a further four eggs obtained from dissected females, gave a total of 10 eggs to be used in the captive incubation experiment, which was carried out in the following manner. Five hundred ml of a 1 p. p. m. potassium permanganate solution was placed in a plastic container measuring 25 x 16 x 8 cm. This solution was used to sterilize the environment in which the eggs were located to reduce the risk of fungal contamination. Then a metal mesh measuring 25 x 16 x 8 cm was placed in the container and on this mesh was placed a covering of moss (*Porotrichum*: Neckeraceae). The moss was dampened with the potassium permanganate solution and after weighing the eggs were placed on the moss and covered with a transparent plastic sheet secured with a plastic band. Temperature and relative humidity were measured using a Schulties thermometer and a relative humidity detector, respectively, installed in identical apparatus.

For the incubation experiment, a concrete room measuring 3 x 3 m was used. The room was located within a dwelling 100 m from the creek. The container with the eggs was placed in the bottom of a cupboard to protect it.

During incubation the eggs were observed periodically and if fungal growth was detected the affected eggs were immediately removed. In order to determine the temperature of the natural environment in the creek interior, a Schulties thermometer was placed 20 cm within the interior of a crevasse (without closing the entrance) in which eggs and shells had previously been observed. In a similar way, the environmental temperature was recorded outside the crevasse using a thermometer suspended 20 cm above the ground. Under natural conditions, the temperature was taken throughout the day and part of the night and at the same time the temperature within the interior of the incubator in the dwelling was taken. In the experimental incubator the temperature was taken throughout the day and night.

In order to register environmental conditions at the study site, a permanent pluviometer was installed. In addition, a thermometer recording maximum

and minimum temperatures was employed and a relative humidity detector was used to record the relative humidity during the days of the study. The closest meteorological station is located at Santiago de Puriscal, 16 km from the creek at an altitude of 850 m above sea level. The dry season corresponded to the months of December to April (< 300 mm of precipitation) and the rainy season from May to November (> 300 mm of precipitation).

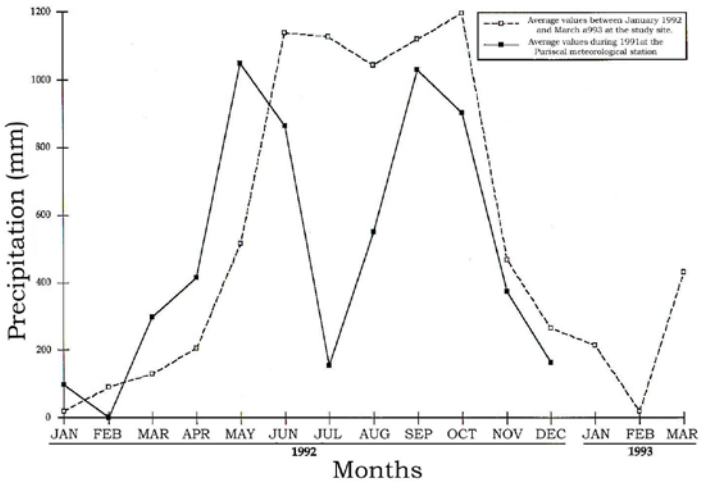

Figure 2. Average monthly rainfall in 1991 at the meteorological station in Puriscal, and from January to December 1992, and January to March 1993 in the Palma de Puriscal creek.

A Student's T-test was used to compare the clutch size of one and two eggs of the *A. aquaticus* females. Correlation and linear regression were used to examine the relationship between body size and testicles, and between body size and dewlap size. Chi-squared was used to compare females that laid one and two eggs per clutch (Zar, 1999). The analyses were carried out using the statistical program SPSS.

RESULTS

The two study years showed somewhat different average monthly rainfall, with peaks in May to September in 1991 (average annual value 2103 mm) and in June to October in 1992 (average annual value 2279 mm; Fig. 2). The average monthly relative humidity values inside and outside the creek were variable during the months of the study (Fig. 3). Similarly, the average monthly maximum, minimum and median temperatures in the study site in 1992 and January to March of 1993 were variable (Fig. 4).

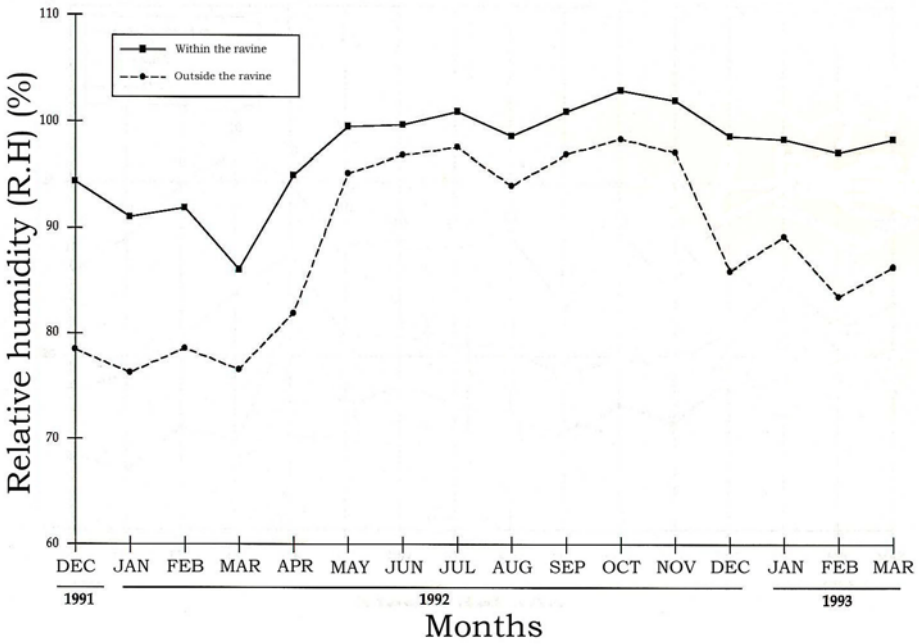

Figure 3. Average monthly relative humidity December 1991, January to December 1992 and January to March 1993 in the Palma de Puriscal creek.

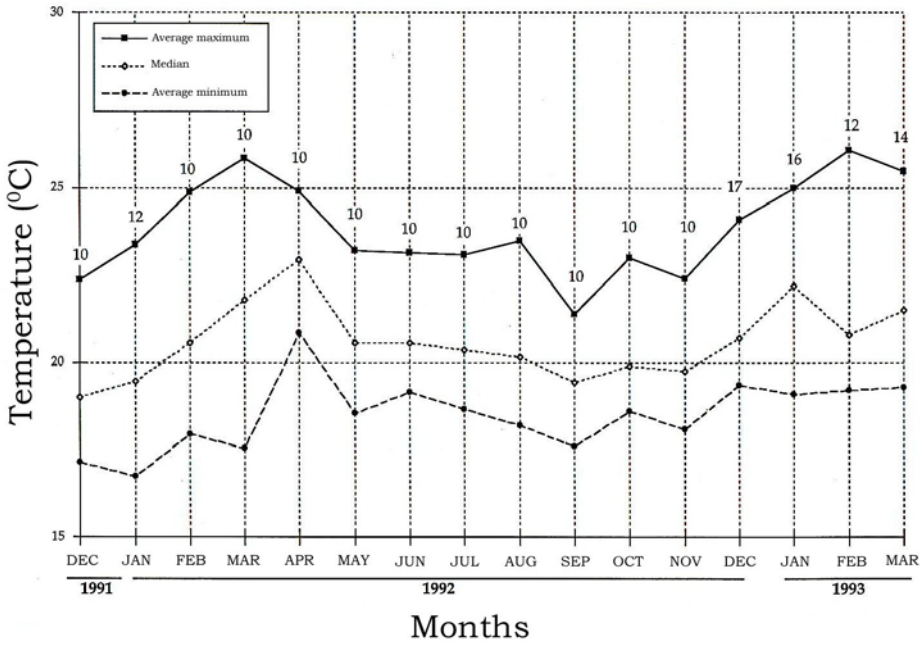

Figure 4. Average maximum, minimum and environmental temperatures December 1991, January to December 1992 and January to March 1993 in La Palma de Puriscal creek.

Sexual maturity of male and females.- Twelve males with SVL of between 38.20 and 77.22 mm were dissected. Males measuring less than 50.02 mm in SVL were found to have testicles measuring 1.5 - 2.5 x 1.4 - 1.9 mm and their seminal tubes (measuring 5 - 5.9 x 0.05 x 0.10 mm), present only as fine light brown lines, were not well defined and were located above the kidney. Hemipenes were not observed (Fig. 5a-f).

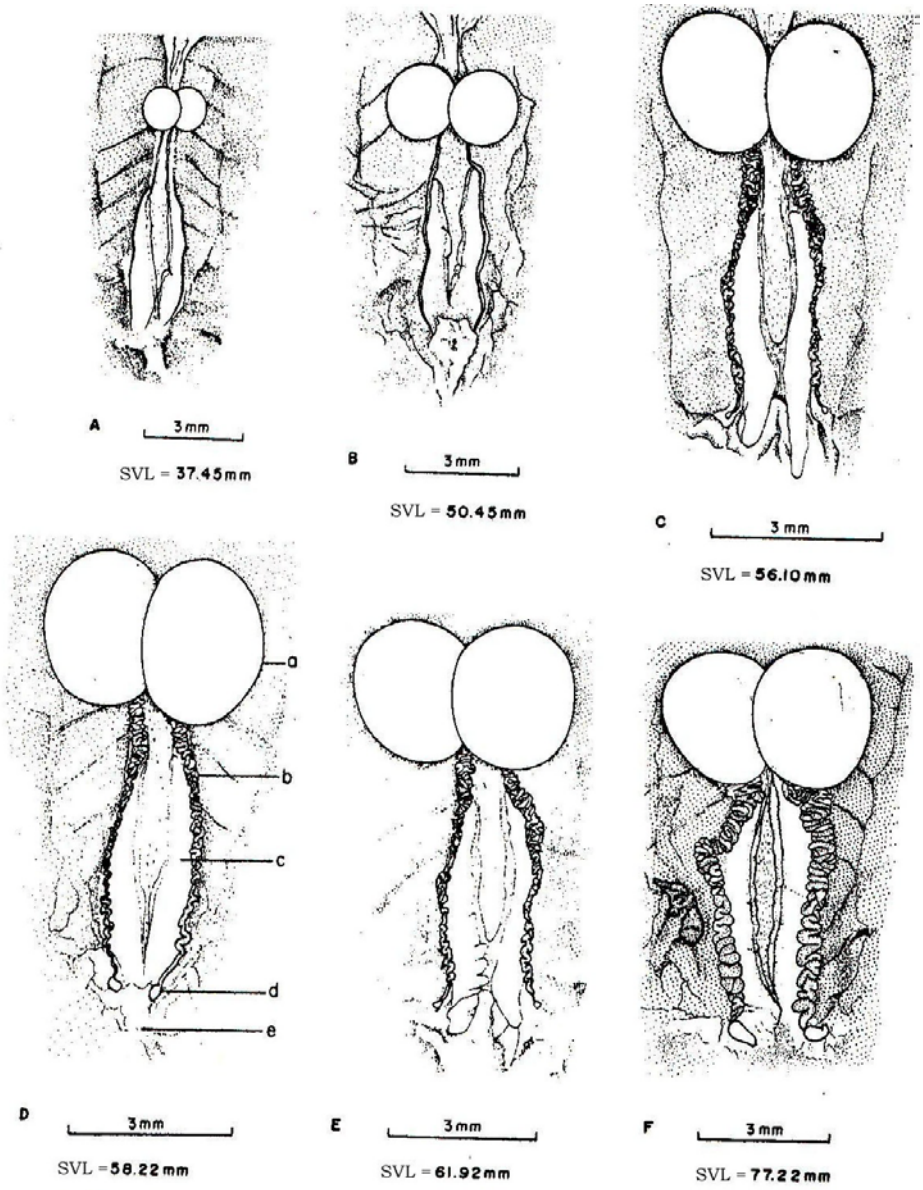

Figure 5(a-f). Sexual maturity of the gonads in *Anolis aquaticus* males. (a) Testicles, (b) seminal tube, (c) kidney, (d) hemipenes, (e) cloaca.

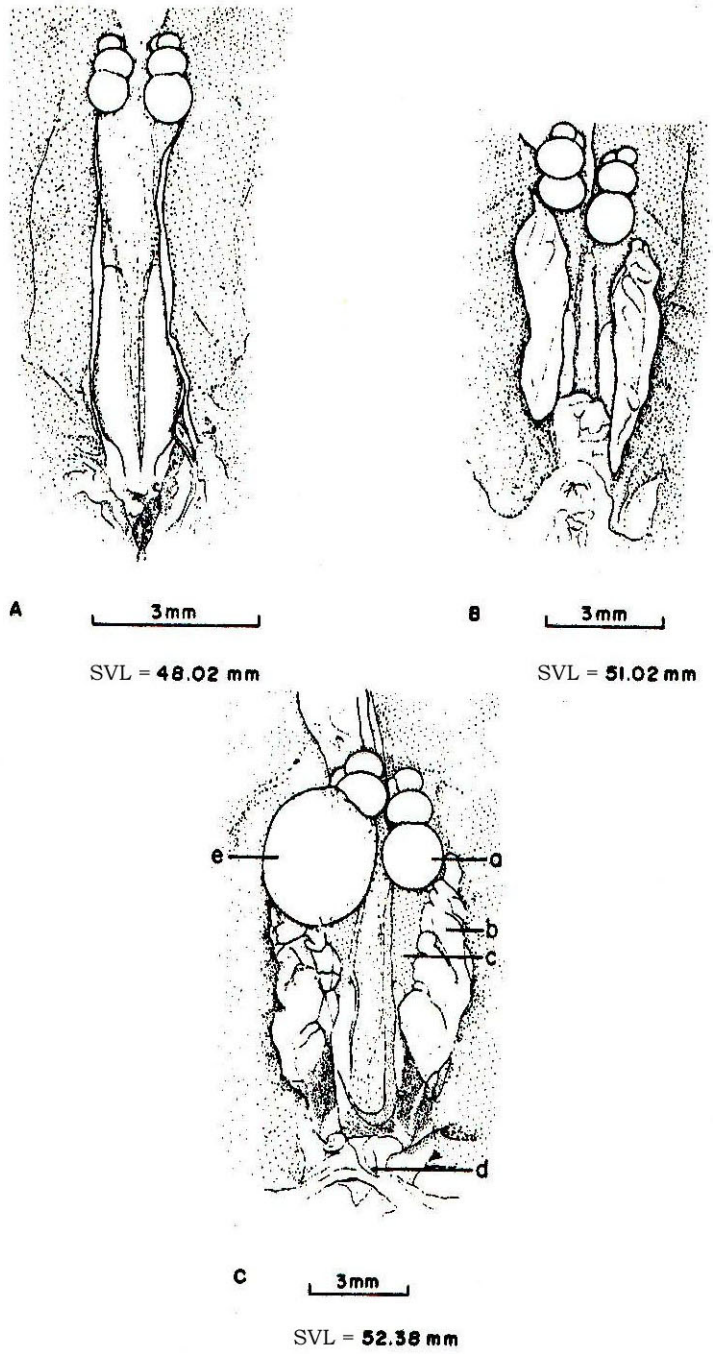

Figure 6(a-e). Ventral view of the gonads of a female *Anolis aquaticus*. (a) Immature follicles, (b) oviduct sac, (c) portion of the kidney, (d) cloacae, (e) mature follicle.

Females gonad size.- The range in SVL of the 11 dissected females was from 36.7 to 65.8 mm. In females less than 48.02 mm in SVL the oviducts were not

yet defined. All that could be seen was a transparent white line extending along the kidney, and the sizes of these oviducts were 5.8 - 6.2 x 0.6 - 1.1 mm. The size of the ovaries was 1.2 - 1.8 x 0.6 - 0.9 mm, and each was composed of 3 - 4 lobes (Fig. 6a-c). The gonads of females with SVL of 52.38 - 65.82 mm were generally well developed. Their ovaries consisted of 3-4 lobes and one or two eggs were always found in the process of formation. Females with SVLs of 48.03 - 51.30 mm were not dissected (Fig. 6a-e).

Males gonad size.- Examination of the gonads of the 12 males each with SVLs greater than 38 mm revealed the presence of testicles without vestigial hemipenes.

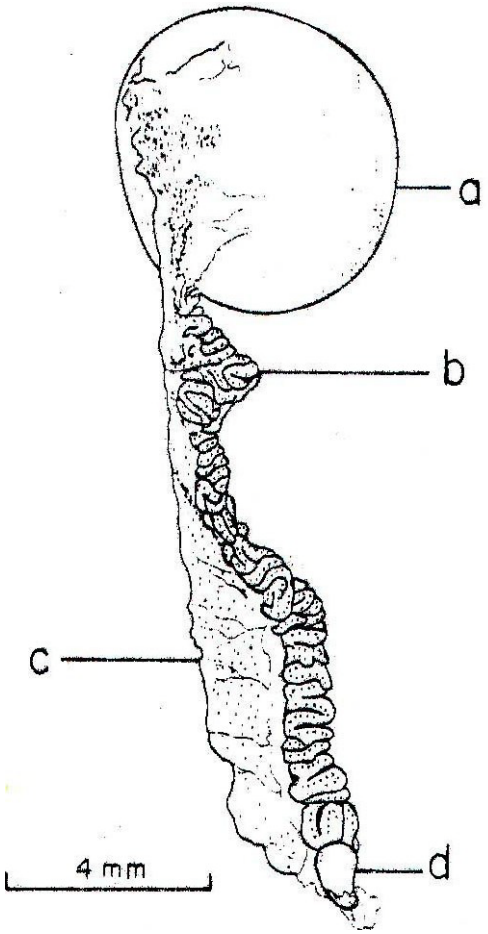

Figure 7. Length of the testicles in relation to body length in *Anolis aquaticus* males.

The dissected males measured between 38 and 56.10 mm in SVL. In males with SVLs less than 50 mm the hemipenes were not clearly defined, all what was observed was a white line extending the length of the kidney. The sizes of the testicles were 4.8 - 3.4 mm in diameter in a male with a SVL of 56.10 mm. In this male the seminal tubes measured 7.2 x 0.4 mm and were serpentine in appearance and light brown in colour with the hemipenes at the

extreme apex (Fig. 7a-d). In this animal, as in other animals greater than 56 mm in SVL, a white liquid was extracted from the hemipenes that was considered to be seminal fluid, based on the information that the urine of these animals is clear. The size of the testicles corresponded to 6.9 - 8.8% of the body length of the animal was also observed. A direct proportional relationship was found between body length and testicle length in this species which was found to be statistically significant ($r = 0.98$, $p < 0.001$; Fig. 8).

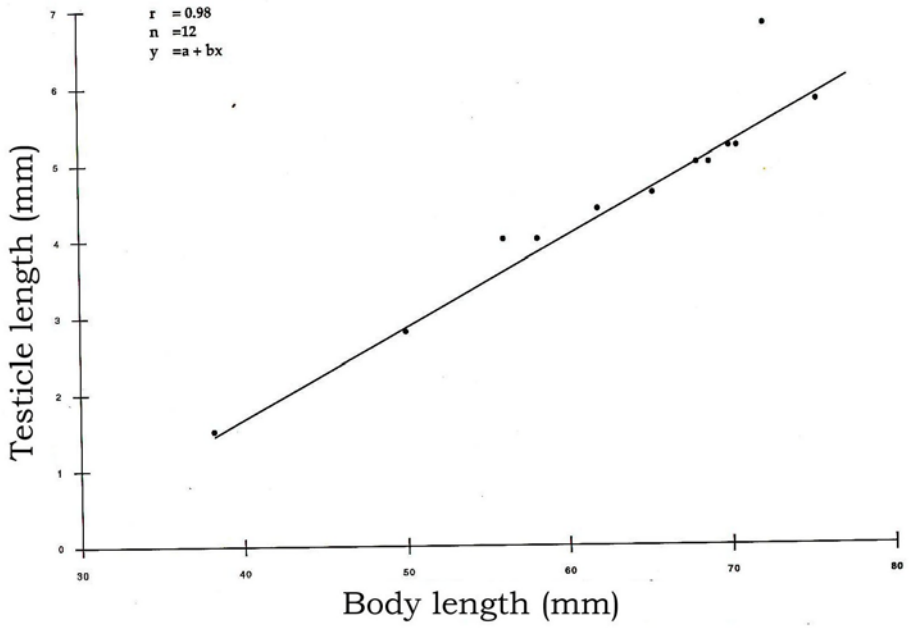

Figure 8. Testicle length related to the body length of the *Anolis aquaticus* males

Dewlap Display.- Males position themselves in the most relevant location of their territory and on several occasions flexes were observed in the presence of other males, juveniles and small terrestrial birds such as the buff-rumped warbler (*Phaeothlypis fulvicauda*). Once the female is sighted, it flexes his front legs three to six times within the space of 15 seconds, balancing his body above and below, and approaches the female. At the same time it displays the dewlap two or three times. If the female is within his territory, the male moves towards her, balancing his body; then he moves in circles around her at intervals from two to six seconds continuing the balancing of its body. Later the female apparently tries to escape from the male with zig-zag movements or circling within his territory.

The maximum width of the extended dewlap corresponded to an average of 35.4% (8.47 - 42.1%) of the animal's body length. A direct proportional relationship existed between body length and dewlap size which was found to be statistically significant ($r^2 = 0.91$, $p < 0.001$; Fig. 9a-b).

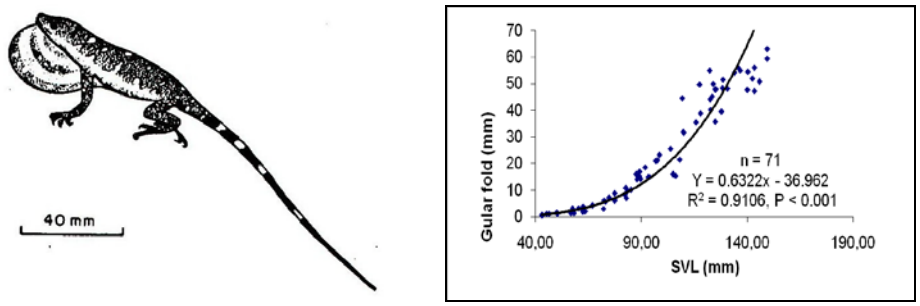

Figure 9. a-b) *Anolis aquaticus* male displaying the dewlap in La Palma creek. b) Relation between body length and dewlap width at maximum extension in anole males (*Anolis aquaticus*) in La Palma de Puriscal creek, San José, Costa Rica.

Mating.- It was observed that for *A. aquaticus* prior to courting and copulation, the female approaches the male's territory where it is balancing its body and displaying its dewlap. *A. aquaticus* females do not approach one another; each of them has their own territory within the territory of the male. A female never tolerates the approaching of another female to a distance of less than 2 m, and if by chance two females find themselves closer than this distance, the female that has a longer history in the aforementioned territory will displace the one with the shorter history. The territory of a male can contain up to two adult females and several immature juveniles that enter and leave the territory. Mating occurs all year round, but greater frequency was observed in the warmer season.

Courting, mounting and copulation.- Courting and copulation were observed on only three occasions; two complete copulations and one incomplete copulation. The first complete copulation was observed between 07:00 and 08:00 in April 1992 and lasted 6 minutes 30 seconds. The second was observed between 09:00 and 10:00 in January 1993 and lasted 5 minutes 45 seconds. Complete copulations are those that contained dewlap display, site selection by the female, the male approaching the female, courting, mounting, copulation and separation of the couple. In the incomplete copulation which was registered at 16:50, only part of the copulation and separation of the couple was observed. The average SVL of the three males that were observed copulating was $\bar{X}$ = 71.17 mm (with a range of 65 - 78.80 mm in SVL), and the value for the females was $\bar{X}$ = 59.22 mm (with a range of 55.59 - 62.76 mm in SVL).

Description of copulation.- The sequence of copulatory events is shown in Figure 10. The females positioned themselves close to a small quantity of flowing water. The males were at a distance of approximately 2 to 2.5 m from the rear of the female, and for 10 seconds the males carried out a dewlap display. After 40 seconds, the males approached the females from behind until the head was leveled with the flank of the females, and they maintained themselves in this position for a period of 32 to 35 seconds. Then for 30

seconds the males passed its tongue's tip over the flank of the female three times at intervals of 8 - 12 seconds. While the male was carrying out the courting display, the female did not display, only glanced from side to side several times.

Figure 10. Courting and copulation of *Anolis aquaticus* en La Palma de Puriscal creek. (a) The male approaches the female and touches her flank with his tongue. (b) The male advances and locates himself on the left side of the female. (c) The male holds the female with the front and back right legs and inserts his genitals in the female's cloacae. (d) The couple separate and clean their cloacae.

Two seconds later the male rapidly advanced and located himself on the left side of the female. The male held the female in the area of the abdominal armpit using his front and back right legs and placed his tail and back right leg over the female's pelvis. He applied pressure to the tail of the female with the base of its tail until the female raised its, leaving the cloacae exposed and partially open and facilitating the entry of the male genitalia. The couple then joined cloacae and the male carried out insertion of its genitalia into the female's cloacae. For a period of approximately 46 seconds the female balanced her body up and down moving in the same way as the male that remained on its back. Later the male and female remained immobile, occasionally looking from side to side for 90 to 105 seconds until the copulation ended (Fig. 10).

During the final 10 seconds of the copulation, the males carried out side to side movements of the pelvis and tail, and at the same time as these sideward movements retracted the hemipenes (which was deep orange in colour, with one side being inserted in each oviduct of the female) from the interior of the female at which point the couple separated. Both sexes then moved slowly with their tails gently curved in the middle. They then rubbed their cloacae on the ground with the tip of the tail also on the ground and the front part of the body raised, for 30 to 35 seconds. Males and females then moved apart in opposite directions (Fig. 10). While the males made all the movements during mounting and copulation, the females did not make any movement with the exception of the balancing of the body which was carried out in conjunction with the male, and the movement of the head from side to side.

Nesting sites.- Thirty-four eggs were located in crevasses besides small springs, in the rough basaltic rock walls where there are permanently trickling streams of water and among piles of leaves and small sticks. The substrate within the crevasses in which eggs were deposited was smooth because of the presence of clay on the rocks. Inspection of the crevasses along the length of the creek revealed up to six eggs together in the same location.

Quantity of eggs.- Of the 140 females that were examined by touch, 37 (26.4%) were not found to contain perceptible eggs despite having reached sexual maturity according to the pre-established size, having SVLs of between 53.27 and 65.92 mm. A single egg was found in 76 females (54.3%) which had SVLs of between 54.88 and 67.42 mm. Two eggs were detected in 27 females (19.3%) which had SVLs of between 59.69 and 68.22 mm. No difference was observed between the females that had no eggs and those that had one or two eggs ($X^2 = 4.45$, g. l. = 2, $p > 0.10$). No eggs were found in females with a SVL of less than 52 mm (Table 1).

Two females laid two eggs in captivity, and among the 11 females that were dissected, eight (72.73%) had a single egg and three (27.27%) had two eggs (one in each oviduct). This egg were compared with the eggs laid by several

females in natural conditions and in captivity and it was found that externally they appeared similar and when viewed through light the embryos were developing normally. It therefore appears that the egg is fertilized and then passes sometime within the female prior to oviposition.

Table 1. Eggs quantity in the female´s oviduct of *A. aquaticus* in the Palma of Puriscal Creek, San José, Costa Rica.

Touch eggs	n/N	%	SVL Range (mm)	Weight Range (g)
0	37/140	26.43	53.27 – 65.92	2.8 – 5.8
1	76/140	54.29	54.88 – 67.42	4.0 – 6.2
2	27/140	19.28	59.69 – 68.22	5.0 – 9.4

n = Sample; SVL = Snout Annus Length; N = Population

On removal of the egg from the oviduct of the eight females, they lost an average of 13.7% of their body weight (range 11.5 to 16.7%); on removal of the two eggs from the oviducts of the other three females, their body weight was reduced by an average of 21.5%. Similarly, when females in captivity were weighed before and after laying, two females that each laid a single egg lost an average of 13.8% of their body weight, while four females that laid two eggs lost an average of 21.7% of their body weight (range 17.6 to 24.0%). Under natural conditions several females were found to lose weight from one month to the next or between one capture and the next (Fig. 10).

Shape, colour and size of the eggs.- The eggs are oval in shape, though some were more elongated than others, with an average length of $\bar{X}$ = 15.32 ± 0.89 mm (R = 14 - 17.7 mm, n = 26) and a maximum width of $\bar{X}$ = 8.34 ± 0.96 mm (6.9 - 10.5 mm, n = 26) (Fig. 11). The weight of the eggs varied between 0.6 and 1.0 g with $\bar{X}$ = 0.7 ± 0.11 g. The shells were soft and white in colour with the flexibility of parchment. When the females had two eggs in their oviducts, at laying the eggs weighed less ($\bar{X}$ = 0.65 ± 0.08, R = 0.6 - 0.8, n = 14) than when females laid single eggs ($\bar{X}$ = 0.78 ± 0.13, R = 0.7 - 1.0, n = 9). There was no statistical difference between the weights of the eggs coming from females who had only one egg in their oviducts and those which had two (t = 1.68, g. l. = 21, p > 0.05).

Incubation.- The 11 females that were kept in captivity for 30 days laid six eggs. These plus the four eggs originating from the dissected females were incubated in semi-natural conditions. They were located in a plastic container and incubated at an average minimum temperature of 22.4°C (04:00 - 07:00) and an average maximum temperature of 25°C (at 15:00). The peak temperatures under natural and semi-natural conditions occurred at different times (Fig. 12). In the semi-natural incubation, the relative humidity was 100% and the average temperatures were between 22.4 and 25°C during the 24 hours of the day. The incubation time was 75 days for a female neonate and 82 days for a male. The two neonates represented a hatching success of 20%.

The fungus *Fusarium solanum* (Moniliacea) was detected on the rest of the eggs.

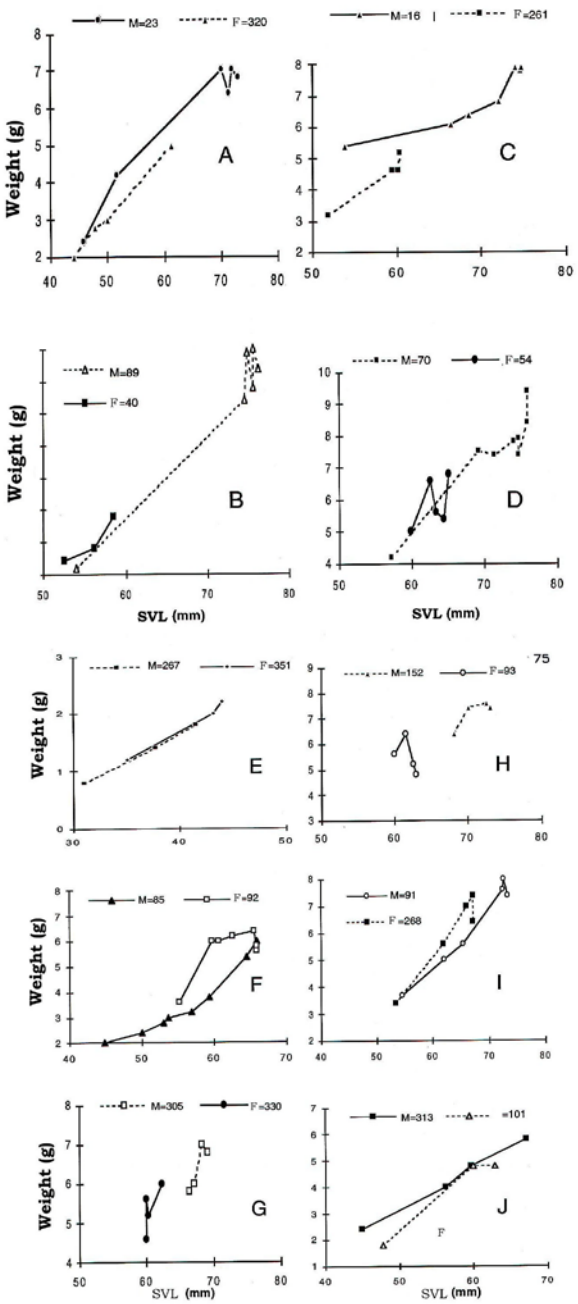

Figure 11. Relation between snout to vent length and weight of the 10 male and 10 female *Anolis aquaticus* most frequently recaptured in La Palma de Puriscal creek. Each graph corresponds to one male and one female (M = male, H = female).

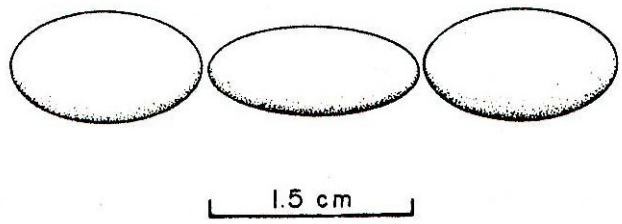

Figure 12. Shape and size of *Anolis aquaticus* eggs.

Hatchling size.- Two neonates (one male and one female) were hatched in captivity, each with a SVL of 25.3 mm and a weight of 0.7 g. The length of the female's tail was 38.32 mm and that of the male was 37.24 mm. In the wild only two individuals of this size were captured (both 26 mm in SVL and 0.6 g in weight). According to the weight loss and size increase of the neonates during nine days of captivity, it is estimated that the young captured in the field were between approximately nine and 12 days of age.

Growth rates of the young.- After hatching, both hatchlings were kept enclosed in the same incubator for nine days and were offered small butterflies and flies as food. However, neither of them ate. In nine days the female grew 2.5% in SVL and the male grew 1.5% in relation to their initial size. The female had a growth rate of 0.07 mm/day and the male 0.04 mm/day. Both animals lost 14.29% of their initial weight during the nine days of observation.

Of the 76 animals captured in the field, 22 (12 males and 10 females) measured less that 32 mm in SVL when they were captured for the first time. On examination of the gonads of a sample of 22 individuals, it was found that females reach sexual maturity at a size of 52 mm in SVL and a calculated age of between four and six months. Males were found to reach sexual maturity at a size of 54 mm in SVL and an estimated age of five to seven months.

In the field 14 juveniles were recaptured three to six times. The juvenile females less than 52 mm in SVL were found to have growth rates of $\bar{X} = 0.11$ mm/day and 0.02 g/day. The juvenile males less than 54 mm in SVL were found to have growth rates of 0.23 mm/day and 0.02 g/day. These rates were greater than those demonstrated by the two neonates in captivity. The growth rates of the juvenile males were significantly greater than those of the juvenile females ($t = 2.39$, g. l. = 56, $p < 0.01$).

Recruitment (Addition of new individuals to the transect in the creek where monthly inspection was carried out).- The addition of young males to the population was 37% and that of females was 25.4%, during the 17 months of the study. A difference was observed between the percentage of addition for males and females ($X^2 = 3.89$, g. l. = 1, $p < 0.05$). The probability of repeat capture of an individual in the study area was 51.6% for juvenile males and

40.1% for young females. The minimum stay for young males and females was one month (two recaptures). The maximum number of recaptures was eight during 17 months for males (with a maximum lapse of 17 months between the first and last capture) and seven times for females (with a maximum lapse of 15 months between the first and last capture). A number of predators were present that limited recruitment of juveniles after hatching, like for example anole lizard adults of the same species that were observed eating young hatchlings. In addition, river crabs (*Ptychophalus tristanis*) and snakes such as *Chironius grandisquanis* and *Leptodeira septentrionalis* were also seen eating anole juveniles and adults.

DISCUSSION

Sexual maturity of males and females.- After having been born, *A. aquaticus* females arrive at sexual maturity in an estimated four to six months (> 52 mm in SVL) (this estimate is based on the growth rates of animals in the field). Males achieve sexual maturity at an estimated five to seven months after birth (> 54 mm in SVL). Other species such as the semi-aquatic lizards *A. lionotus* and *A. poecilopus* reach sexual maturity more rapidly at between two and three months after hatching (Campbell, 1973).

The length of the testicles in *A. aquaticus* males has a direct proportional relationship with body length. The seminal tube was observed to be serpentine in shape and when stretched out its length is close to the body length of the animal; in addition the testicles of this species were large in relation to their body. Rueda (1989) found that males of *A. megalopithecus* had spherical testicles.

Dewlap display.- Throughout disputes and territorial interactions with other males and with females, the males repeatedly extend and contract the dewlap and move the head and body up and down. In this behavior the species is similar to various other species of *Anolis* and *Norops* as mentioned in the introduction. It was always the intruder who abandoned the conflict and withdrew. In several species of anole in Cuba, the males extend the dewlap, compress their bodies laterally and elevate their extremities to appear larger than they actually are (Rodríguez & Larramendi, 2003).

Courting, mounting and copulation.- In the two complete and one incomplete copulations observed it was noted that the receptive females permitted the males to approach their backs. The dewlap display of the male prior to approaching the female, and the passing of the tongue of the male over the pelvic region of the female could be considered as pre-copulatory courting that the male uses to allow copulation to continue and to get post-copulatory responses from the female that favour the reproduction of the male. Hews (1990) states that female lizards are receptive for a relatively short time (24 - 48 hours). Rodríguez & Larramendi (2003) report that in captivity *A. inexpectata* males initiate courting with various rapid extensions of the

dewlap accompanied by short and quick nods and if the female is receptive it too responds with short nods.

In the three couples observed, the males did not carry out post-copulatory courting. The *A. aquaticus* copulation lasted 90-105 seconds, while that of several species in Cuba has duration of 5 to 13 minutes, depending on the species (Rodríguez & Larramendi, 2003). The way in which the male approaches the female and whether he embraces her with the front and back right legs or the front and back left legs depends on the species (Andrews & Asato, 1977; Rodríguez & Larramendi, 2003). *A. aquaticus*, *A. allisoni*, *N. polylepis* and *A. bartchis* mount females using the front and back right legs. In contrast, *A. bremeri* and *A. sagrei* mount females using the front and back left legs. Both males and females clean their cloacae by rubbing on the ground after completing copulation and separating. Molina (1981) in a study of *Gallotia galloti* and Hirth (1963) in observations of a species of *Ameiva,* both report that male and females both clean the cloacae after defecation or copulation.

Laying and incubation of the eggs.- The nesting of *A. aquaticus* in crevasses where there is a permanent small trickle of water could be due to the requirement of the eggs for a smooth and very damp substrate. Such sites allow the water potential balance between the substrate and the eggs to remain constant and in this way desiccation is avoided. Although a number of other species of *Anolis* and *Norops* live in trees, the females always have to descend to lay their eggs between fallen leaves, in holes in the trunks or in excavated holes in the ground (Andrews & Asato, 1977). The eggs of Caribbean anoles are leathery in texture and are deposited below very damp fallen leaves where they are incubated by the environmental heat (Rodríguez & Larramendi, 2003).

According to the results of the examination by touch of females and observations of the presence of neonates on the transect, it was concluded that *A. aquaticus* reproduces all year round with a small reduction at the start of the rainy season, the months May to June and part of July. Females can lay one or two eggs at intervals of a month or more; the proportion of females with eggs in their oviducts is lower during the rainy season. According to Andrews & Asato (1977), in other locations in Central America, in species of similar size, females that were repeatedly captured and examined by touch during the dry season laid one egg every two weeks, and the rate of oviposition could increase to one egg per week during the rainy season. Females that lay every month were less that 60 mm in SVL. After laying, females with a SVL of greater than 60 mm had very slow recuperation and laid every 45 to 60 days. The majority of the animals measuring 63 or 64 mm in SVL did not recover and became very thin and several died (Márquez, 1994; Márquez *et al*., 2005). In the Caribbean, female *N. polylepis* and *A. argenteolus* lay eggs throughout the year with an emphasis in May and

September. In many species, juveniles are found to be abundant between July and September (Rodríguez & Larramendi, 2003).

None of the females with a SVL of less than 52 mm (7.9%) had eggs when they were captured and examined by touch and it is supposed that at such a size they are sexually immature. A number of females greater than 53 mm in SVL also lacked developed eggs when examined by touch. It is assumed that these females had nested recently and the next egg was in the process of maturation.

Figure 13 shows the average incubation temperature of the eggs of *A. aquaticus* under natural conditions inside and outside crevasses in the creek. This difference in the temperature profile during incubation suggests that the incubation time would be better (between 85 and 90 days to hatching) for eggs within crevasses in the creek where the monthly average temperatures are lower in comparison to those observed in semi-captivity where the neonates that hatched took 75 to 82 days to hatching. In reptiles, high temperatures reduce incubation time and low temperatures increase incubation time (Márquez *et al.*, 1989). The incubation success of the eggs depends on the conditions offered by the nest (Werner & Rey, 1987), with humidity being of extreme importance as substrates that are too dry destroy the eggs (Werner, 1988).

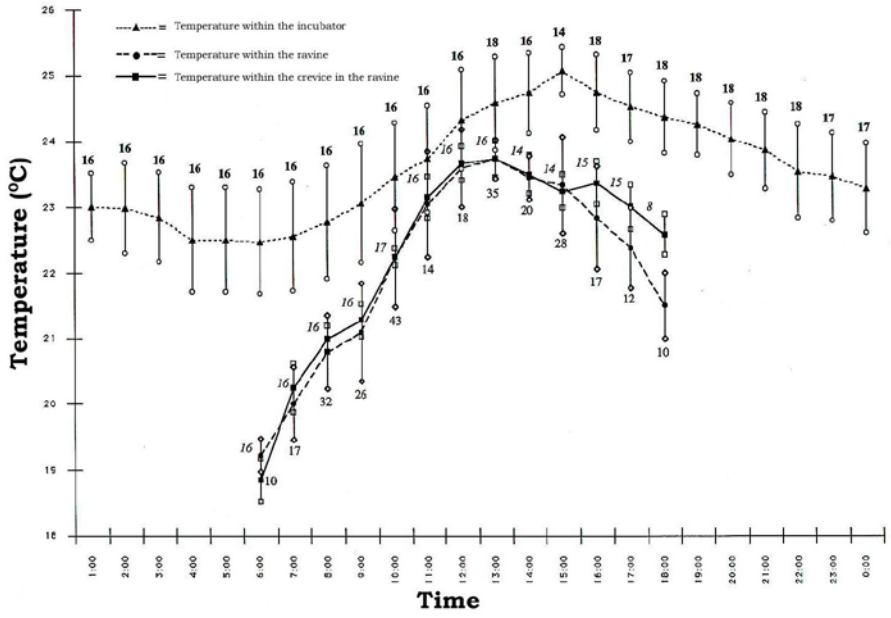

Figure 13. Average temperature within the artificial incubator that contained 10 *Anolis aquaticus* eggs, and within a crevasse in the creek which was used as a nesting site by anoles, during 24 or 12 hours of the day during the incubation period up to the hatching of the young.

Nests with humid subsoil give clutches with larger and heavier eggs (Gordon, 1960). The eggs of *A. aquaticus* females were small and weighed less when laid, and the two eggs that hatched in captivity increased in size and weight during the course of the incubation. According to Werner (1988), the nest conditions influence the size and appearance of recent hatchlings in *Iguana iguana*, with a difference as large as 15% in SVL being observed when eggs were incubated under favorable temperature and humidity conditions when compared with less favorable conditions. Werner's observation can be extrapolated to the *Norops* and *Anolis*. Andrews & Asato (1977) suggest that the incubation period of anoles is around 50 days. In this study the length of the incubation period of *A. aquaticus* eggs in captivity was in the region of 75 days for females and 82 days for males. Few incubation and hatching data were collected in captivity, and for this reason it is not possible to identify relationships between the humidity of the substrate and body length and weight of the offspring on hatching, though it is noted that the temperature of the nest sites in the creek was relatively cool.

In *A. aquaticus,* the size of the hatchlings at birth is no greater than 26 mm in SVL and the weight 0.7 g. The parameter most variable between the sexes was the length of the tail. Growth was at a slower speed when compared to other species of anoles, and sexual maturity was reached at between four and six months for females (when they reached 52 mm in SVL) and five and seven months for males (when they reached 54 mm). The size of the young of *N. polylepis* at birth is 19 mm in SVL, but growth is rapid and they reach sexual maturity at between three and four months, when they reach a size of 39 mm in SVL (Andrews & Asato, 1977).

Growth rates.- If all of the sizes of *A. aquaticus* are considered, average growth rates of between 0.04 and 0.23 mm/day were recorded (with a range of 0.02 to 0.28 mm/day) and average weight gains were between 0.02 and 0.04 g/day. The values recorded for *A. poecilopus* (Cope 1862; 0.30-0.48 mm/month) by Campbell (1973) fall below those recorded in this study. Nevertheless, in the males and females greater than 74 and 64 mm, respectively, the growth rate is slow and approaches zero. In the green iguana, the growth rate in SVL is 0.23 mm/day and reduces with high levels of territorial activity in males and with the laying of eggs in females (Werner & Rey, 1994). The daily growth rates in body length for green iguanas were greater than those obtained for anoles in this study.

The average growth rates of males and females were not different, though juveniles showed a different growth rate than adults. Under natural conditions, anoles present growth rates different than those raised in captivity, though the difference was not found to be significant. The slight difference between the growth rates of the animals in captivity and those in natural conditions could be related to the absence of flowing water that serves as an escape from predators. This species lives at a distance no greater than seven meters from water (Márquez, 1994). According to Savage and Guyer

(1989), *A. aquaticus* is a species that can be found close to flowing water and it enters the water to escape predators. Leal & Lossos (2000) found that the same behaviour followed by *A. aquaticus* has evolved twice in the Caribbean and twice on the Central American continent. It is possible that the capture, manipulation and confinement of these animals were major stresses (Romero & Wikelski, 2001; Márquez *et al.,* 2007).

Males greater than 69 mm in SVL (n = 102) increased in body length from one month to the next, but the lost of their body weight is between 5 and 10%. Females greater than 59mm in SVL (n = 78) also gained body length from one month to the next, but they lose between 13.7 and 21.8% of their body weight, probably due to the laying of eggs. This weight was always regained within days or weeks of laying when the females measured less than 63 mm in SVL. Females greater than 63 mm in SVL (n = 35) did not regain weight after laying and continued to lose weight until they became skeletal and died of hunger. These were probably very old individuals. It is thought that males lose weight due to mating activities, reduction of foraging activity, bad health, environmental effects such as El Niño and lack or food. The females are thought to lose weight due to egg laying, bad health, environmental effects and lack of food. The same behaviour is observed in marine and terrestrial iguanas under natural conditions and in captivity (Wikelski & Thom, 2000; Márquez, unpublished data).

Recruitment.- Anoles males, females and juveniles are preyed by other reptiles such as the snakes *Chironius grandisquanis* and *Leptodeira septrionales*, by adult *Norops* and Anoles and by the river crab *Ptychophalus tristanis*, and all of these were observed eating *Norops*. These organisms limit juvenile recruitment and also the population in general. From hatching to reaching sexual maturity, 60% of *Norops* are lost to predation (Márquez, 1994). In other lizards such as the *Microlophus* of the Galapagos Islands, recruitment can reach between 20 and 40% of the annual litter (Werner, 1978; Stebbins *et al.*, 1967).

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