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Ultrasound Scanning of Ovaries and Eggs in Galápagos Tortoises, *Geochelone nigra*, on Santa Cruz Island, Galápagos

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Abstract. – Ultrasound investigations were performed on 12 adult female Hood Island Galápagos tortoises (*Geochelone nigra hoodensis*) at the Charles Darwin Research Station (CDRS) on Santa Cruz Island, Galápagos, between 1995 and 1996. Ovarian activity and follicular growth were monitored throughout the year. Vitellogenic follicles ranged between 21–42 mm. Preovulatory sized follicles (41–42 mm) were seen in all animals between May and October, coinciding with the end of the mating season (June) and matching the nesting season which starts in May when environmental temperatures decrease. Atretic follicles 8–62 mm were found in all months. The largest atretic follicles were found from February to July during the hot and rainy mating season. Females laid 1–3 clutches (5–12 eggs per clutch, mean = 7.0) during the dry and cool season from June to November. Eggs remained in the oviduct 17–50 days. The interclutch interval ranged 30–64 days. Additionally, 8 adult female Galápagos tortoises of unknown origin (*G. nigra* ssp.) were scanned during the same period of time. All tortoises were kept under semi-natural conditions. Temperatures were highest during the mating season in February and March (monthly means, 24.1–30.8°C) when the heaviest rainfall occurred. Lowest temperatures were measured during the nesting season in August and September (monthly means, 19.8–23.9°C) coinciding with the dry season when *garúa* (drizzle) occurred almost every day. This study elucidates for the first time the annual reproductive pattern of *G. n. hoodensis*, one of the rarest tortoises on earth and confirms that the ovarian cycle of the Galápagos tortoise is seasonal and correlates with the hormonal data that have already been published.

Key Words. – Reptilia; Testudines; Testudinidae; *Geochelone nigra hoodensis*; tortoise; reproduction; ultrasonography; ovarian follicles; oviductal eggs; nesting; environmental cues; semi-natural conditions; temperature; Galápagos; Ecuador

The Galápagos tortoise, *Geochelone nigra*, is a species classified under Appendix I by CITES and designated Vulnerable by IUCN 1996 Red List of Threatened Animals. The populations on the Galápagos Islands have been severely impacted by human exploitation. Eight of the eleven surviving subspecies are threatened due to predation and competition from introduced mammals (MacFarland et al., 1974) and are vulnerable to natural and man-made disaster (Pritchard, 1996).

Knowledge of the reproductive physiology of the Galápagos tortoise remains extremely limited mainly because its threatened status restricts the use of invasive techniques for studying reproductive patterns. Annual hormonal cycles have only been reported for Galápagos tortoises that are kept in zoos (Casares, 1995; Rostal et al., 1998b). We previously reported plasma concentrations of estradiol, progesterone, testosterone, and corticosterone in Galápagos tortoises (*G. nigra* ssp.) at the CDRS (Schramm et al., 1999a) but blood sampling in *G. n. hoodensis* was not possible. With this restriction we were able, however, to monitor the reproductive cycle of this subspecies using ultrasonography. Ultrasound scanning allows direct observation of the reproductive organs without the necessity of using invasive procedures and is free of any radiation side-effects (Baker and Dalrymple, 1978; Kuchling, 1998). Ultrasonography has been applied to chelonian species for evaluating anatomical structures (Penninck et al., 1991; Schildger et al., 1994), follicular activity and clutch formation (Kuchling, 1989; Rostal et al., 1990), as well as for long-term monitoring of the ovarian cycle (Kuchling and Bradshaw, 1993; Rostal et al., 1996, 1997). Only a few ultrasonographic studies have been conducted in female Galápagos tortoises (Robeck et al., 1990; Casares et al., 1997; Rostal et al., 1998b), and all animals in these studies were held in zoological gardens. No data from free-ranging Galápagos tortoises exist. In the present study the annual reproductive cycle of a very rare subspecies of the Galápagos tortoise (Hood Island tortoise, *G. n. hoodensis*), held under semi-natural conditions on Santa Cruz Island, was monitored. Using a portable ultrasound unit the reproductive structures were scanned, and the ovarian cycle, vitellogenesis, and clutch formation were monitored. These are the first data on the reproductive cycle of this rare subspecies. Additionally, environmental cues which are thought to be important parameters influencing reproduction were recorded.

MATERIALS AND METHODS

Between November 1995 and November 1996 twenty adult female Galápagos tortoises (12 *G. n. hoodensis*, 8 *G. nigra* ssp.) were examined monthly using ultrasonography. During critical times, such as ovulation and nesting (i.e., between June and November), examinations were conducted every other week in *G. n. hoodensis*. Additionally, nesting females were scanned the day following oviposition. A portable Aloka SSD-500 real-time ultrasound unit (Aloka Co., Ltd., Tokyo, Japan) with a 5.0 MHz convex-array transducer powered by a Honda EX 350 G portable generator were used. Coupling gels (AQUARIUS 101 ultra gel, Medilab Ltd., Israel; CELAGEL, G. Streuli & Co. AG, Uznach, Switzerland) allowed the transmission of the ultrasound between the probe and the body of the animal. The tortoises were scanned through the inguinal shell opening, immediately anterior to the hind limbs, which allows good visualization of the reproductive organs. To stabilize the animals, they were turned over and placed with the carapace in a car tire. They were scanned on both sides of the shell, allowing examination of both ovaries. No sedatives were used. Developing, preovulatory, and atretic ovarian follicles as well as eggs at various stages of shell deposition were identified and measured. Ovarian follicular activity was evaluated by monitoring the size of the largest group of developing follicles. The oviductal period (number of days between the first detection of oviductal shelled eggs and egg deposition) and the interclutch interval (period of time between two clutches), as well as the retention of eggs (number of days eggs were retained in the oviducts beyond the normal oviductal period) were recorded.

All tortoises had been kept under semi-natural conditions at the Charles Darwin Research Station (CDRS) on Santa Cruz Island, Galápagos, Ecuador, for decades. The animals are housed in three different corrals (each approximately 20 x 55 m) and are exposed to the ambient natural habitat and natural environmental conditions, including nutrition, temperature, photoperiod, light intensity, humidity, and precipitation. The corrals include original vegetation (cactus, bushes, shrubs), artificial water pools, and several nesting sites. Three times a week the tortoises were fed fresh herbs, leaves, and grasses which had been collected in the highlands of Santa Cruz. The animals also actively searched for food within their corrals, foraging on various seasonal plants including leaves, flowers, and fruits. During 5 to 6 days a week the tortoises had access to water *ad libitum*. When it rained, mud pools naturally formed and were used abundantly by the animals.

Between 1963 and 1974 the last surviving 14 individuals of *G. n. hoodensis* (2 males and 12 females) were removed from their native island (Española [Hood Island]) to save them from extinction. In 1977 an additional adult male of the same subspecies from the San Diego Zoo was added to the group. For the last 30 years these Española tortoises have been part of the captive breeding program of the CDRS and the Galápagos National Park Service (GNPS) on Santa Cruz Island. Successful reproduction in this group has been recorded since 1971 (Cayot and Morillo, 1997). The 12 adult females housed at the CDRS were investigated in this study.

Another group of 8 females of *Geochelone nigra* ssp. is kept in a separate enclosure at CDRS. These tortoises are of unknown origin from various Galápagos islands and belong to several subspecies not yet identified. These 8 females were examined at monthly intervals.

Seasonal changes of the sizes of developing follicles were determined using a one factor repeated measures ANOVA. Significant changes of mean sizes of follicles between months were validated by a Scheffé's F-test. Individual follicle sizes were subjected to a factorial ANOVA. Significant changes of mean sizes of follicles between

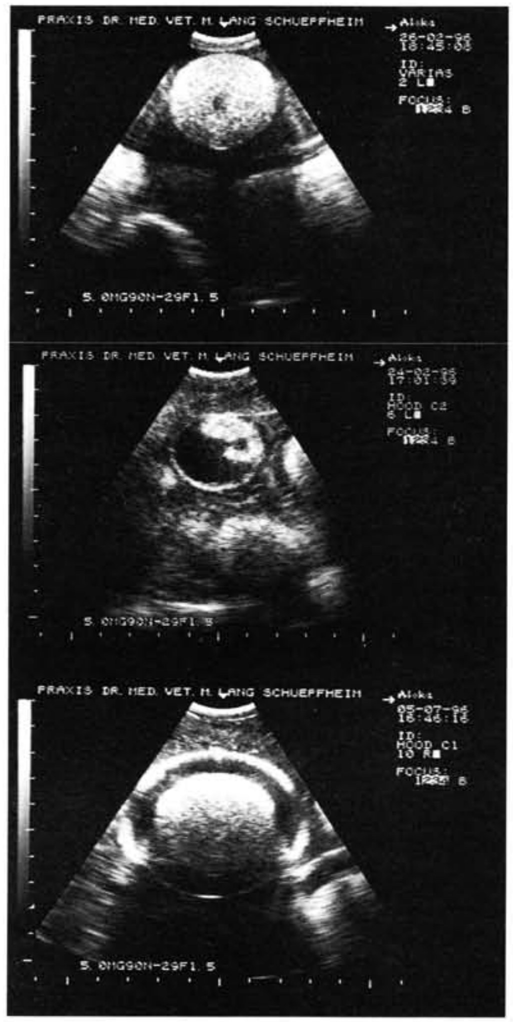

Figure 1. Ultrasound images from adult female Galápagos tortoises (*G. nigra*). Horizontal and vertical reference scales represent 1 cm increments. **Top:** Preovulatory-sized follicle. **Middle:** Atretic follicle with a non-echogenic center. **Bottom:** Thin-shelled egg with a highly echogenic shell, a non-echogenic albumin layer, and echogenic yolk.

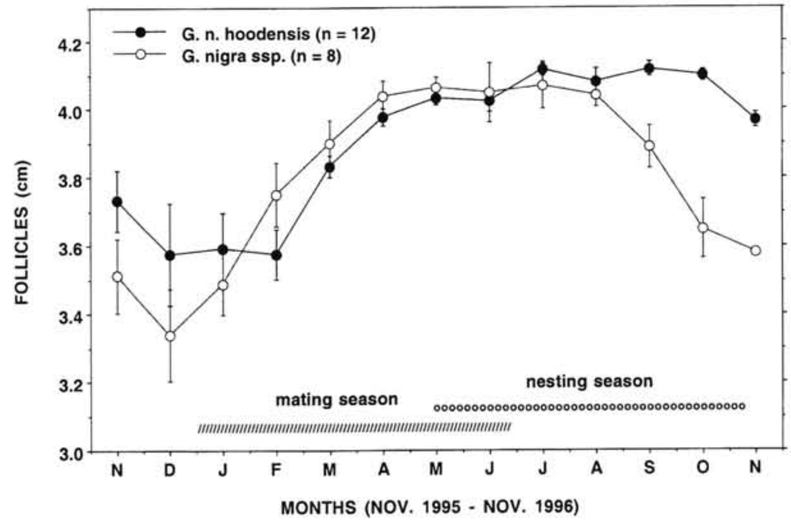

Figure 2. Annual sizes of the largest group of follicles from 20 Galápagos tortoises living under semi-natural conditions at the CDRS.

animals were validated by a Scheffé's F-test. Significance was assumed when $p \leq 0.05$. Analyses were done using StatView software (Abacus Concepts, Calabasas, CA).

Daily changes in air temperature were monitored using a digital meter (UNITEST No. 93420 D, CH. BEHA GmbH, 79286 Glottertal, Germany) with a precision of ± 0.8°C.

RESULTS

Vitellogenic follicles are echogenic and are identified by ultrasonongraphy as spherical and dense structures (Fig. 1, top). Unlike these, atretic follicles show an irregular density and consist of both echogenic and non-echogenic material (Fig. 1, middle). Eggs are spherical or slightly oval in shape and are identified in the oviducts by their highly echogenic shell which is followed by a non-echogenic albumin layer and an echogenic yolk (Fig. 1, bottom).

In the group as a whole (20 females), 98.2% ($n = 605$) of examinations have clearly shown reproductive structures in at least one of the ovaries or oviducts. In 1.8% ($n = 11$) of examinations no follicles could be visualized in one of the ovaries. This was caused by a large number of eggs in the oviducts which completely blocked visibility of other reproductive structures. In 95.5% ($n = 588$) of the cases vitellogenic follicles were observed in either one or both ovaries; 1.0% ($n = 6$) of examinations showed only atretic follicles in one of the ovaries.

Large follicles ranged between 21 and 42 mm and were found in all females throughout the year. The sizes of the follicles of *G. n. hoodensis* females increased significantly (df = 11, 144, F = 3.3, $p \leq 0.05$) between February and July, coinciding with the mating season and the rainy season (January–June) when environmental temperatures were high (mean ± SE between 21.3 ± 0.2°C and 30.8 ± 0.2°C) (Fig. 2). Changes of mean sizes of follicles between animals were not significantly different (df = 11, 144, F = 1.08, $p \leq 0.05$). Largest preovulatory follicles were found from May to October (monthly means 40.3–41.2 mm) during the nesting season when temperatures were low (between 19.8 ± 0.3°C and 27.6 ± 0.2°C). In *G. nigra* ssp., the sizes of the follicles increased significantly (df = 7, 88, F = 4.6, $p \leq 0.05$) between

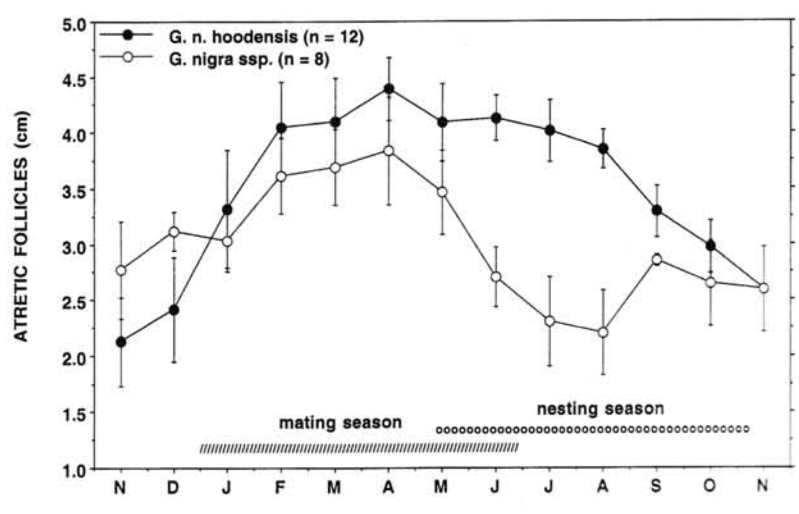

Figure 3. Annual sizes of the largest group of atretic follicles from 20 Galápagos tortoises living under semi-natural conditions at the CDRS.

Table 1. Oviductal period, interclutch interval, clutch size, and date of egg laying from 12 Galápagos tortoises (*G. n. hoodensis*) recorded during one year at the CDRS on Santa Cruz Island, Galápagos.

Tortoise ID	Date of Ovipositon	Time Interval of Shell Deposition	Oviductal Period	Interclutch Interval	Number of Eggs
4	1st clutch: 2.8.96	5.7.–2.8.96	28 days		8
	2nd clutch: 26.9.96	22.8.–26.9.96	35 days	55 days	10
30	1st clutch: 15.8.96	5.7.–15.8.96	41 days		6
1	1st clutch: 25.7.96	18.6.–25.7.96	37 days		7
	2nd clutch: 15.9.96	8.8.–15.9.96	38 days	52 days	7
2	1st clutch: 15.6.96	28.5.–15.6.96	18 days		6
	2nd clutch: 2.8.96	5.7.–2.8. 96	28 days	48 days	5
10	1st clutch: 9.8.96	5.7.–9.8.96	35 days		5
	2nd clutch: 8.9.96	22.8.–8.9.96	17 days	30 days	6
	3rd clutch: ?	23.9.–?.11.96	?	?	?
11	1st clutch: 15.7.96	17.6.–15.7.96	28 days		5
	2nd clutch: 8.9.96	9.8.–8.9.96	30 days	55 days	6
3	1st clutch: 10.8.96	4.7.–10.8.96	37 days		5
	2nd clutch: 23.9.96	23.8.–23.9.96	31 days	44 days	9
6	1st clutch: 10.7.96	17.6.–10.7.96	23 days		12
	2nd clutch: 12.9.96	9.8.–12.9.96	34 days	64 days	9
12	1st clutch: 10.7.96	17.6.–10.7.96	23 days		8
	2nd clutch: 17.8.96	24.7.–17.8.96	24 days	38 days	7
7	1st clutch: 12.9.96	24.7.–12.9.96	50 days		6
20	1st clutch: 8.7.96	27.5.–8.7.96	42 days		7
9	1st clutch: 17.8.96	4.7.–17.8.96	44 days		6
		Mean ± SD:	32.15 ± 8.76 days	48.25 ± 10.75 days	7.0 ± 1.86
		Range:	17–50 days	30–64 days	5–12

December (end of the dry season) and the rainy season (January–June) showing a similar pattern to that of *G. n. hoodensis*.

Atretic follicles were observed during the entire year in all females, usually displaying similar sizes as vitellogenic follicles (Fig. 3). Most follicles undergoing atresia ranged between 8 and 45 mm (86.3%, $n = 170$) but a few structures showed maximal diameters between 46 and 62 mm (13.7%, $n = 27$). In *G. n. hoodensis* largest atretic follicles were found during the mating season between February and July (monthly means 40.1–44.9 mm) when temperature ranged between 20.7 ± 0.2°C and 30.8 ± 0.2°C. In *G. nigra* ssp. largest atretic follicles were observed between February and May (monthly means 34.6–38.3 mm). No post-ovulatory follicles (corpora lutea) were identified in any of the females examined.

Most copulations were observed between January and June (mating season) which coincided with the hot rainy season. During the entire year only seven complete copulations were observed. Most mating attempts were unsuccessful ($n = 269$) because females behaved reluctantly or because males were not able to find the optimal position on the female's back.

At the CDRS, tortoises generally nest from May to October. Female *G. n. hoodensis* laid 1–3 clutches between June and November when temperature was low (between 19.8 ± 0.3°C and 26.0 ± 0.7°C) (Table 1). Clutch size ranged between 5 and 12 eggs (mean ± SD = 7.0 ± 1.86, $n = 20$). The oviductal period ranged from 17 to 50 days (mean ± SD = 32.15 ± 8.76, $n = 20$). The interclutch interval varied between 30 and 64 days (mean ± SD = 48.25 ± 10.75, $n = 8$).

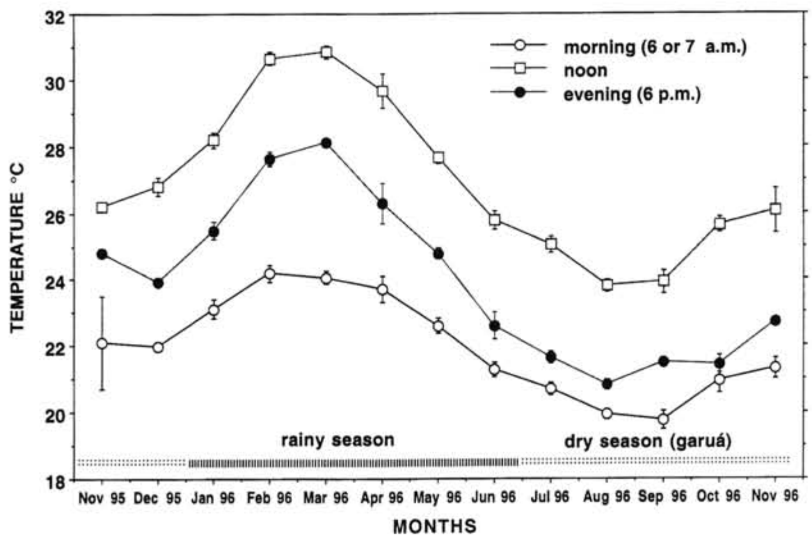

Figure 4. Annual temperature patterns on Santa Cruz Island measured at 0600 or 0700, 1200, and 1800 hrs in the corral of the *G. nigra* ssp. tortoises at the CDRS.

Female *G. n. hoodensis* always laid complete clutches throughout the whole nesting season and did not retain eggs for an extended period of time.

Several females of *G. nigra* ssp. displayed protracted or abnormal nesting activities due to a lack of appropriate nesting sites. However, four individuals which dug real nests produced 1–2 clutches (5–14 eggs per clutch, $n = 7$) between May and October during the dry and cool season. These females showed an oviductal period ranging between 8 and 74 days ($n = 7$) and an interclutch interval between 40 and 89 days ($n = 3$).

The highest temperatures were measured during the mating season in February and March when heavy rainfalls may occur and showed monthly means of 24.1–30.8°C (Fig. 4). Lowest temperatures were recorded during the dry nesting season in August and September when monthly means were 19.8–23.9°C. On Santa Cruz Island (latitude 0°30'S – 0°45'S) annual day length varies approximately ± 30 min. The sun rises between 0515 and 0545 hrs and sets between 1745 and 1815 hrs.

DISCUSSION

Although the Galápagos Islands are close to the Equator, seasonal changes in temperature and rainfall occur. The giant tortoises on these islands have evolved a seasonal reproductive cycle with the mating season (January–June) occurring during the hot and rainy season and the nesting season (May–October) occurring during the cooler dry season.

Geochelone n. hoodensis living under semi-natural conditions exhibit an annual reproductive cycle in which initial follicular growth occurs during the hot, wet season. Follicular development is completed in the dry season (November) when temperatures are low. Mating occurs between January and June, which coincides with the hot and rainy season. The largest preovulatory follicles are seen between May and October in the dry and cooler season. Largest atretic follicles are found during the mating season from February to July, which coincides with the rainy season when temperatures are high. In the other tortoises examined (*G. nigra* ssp.), a similar cycle was observed with the largest preovulatory follicles seen between April and August. Largest atretic follicles in this group were observed between February and May.

The ovarian cycle of the Galápagos tortoise has been described in only a few studies (Robeck et al., 1990; Casares et al., 1997; Rostal et al., 1998b; Schramm et al., 1999b). Investigations were all conducted in captive animals and no study has monitored the reproductive physiology of free-ranging females. Robeck et al. (1990) recorded for the first time ovarian follicular structures and oviductal eggs in a colony of Galápagos tortoises at the Gladys Porter Zoo (Brownsville, Texas). Using ultrasonography they found vitellogenic (developing and preovulatory) follicles throughout the year, ranging 18–44 mm in diameter. Casares et al. (1997) studied the reproductive activities of giant tortoises at Zoo Zurich in Switzerland over two years. They measured largest vitellogenic follicles ranging 30–42 mm in *G. nigra*. Another study, which recently was conducted on the Galápagos tortoises at the Gladys Porter Zoo, reported large vitellogenic follicles ranging 28–42 mm (Rostal et al., 1998b). The animals at the Gladys Porter Zoo showed limited follicular growth during the post-nesting/pre-mating season which coincides with the observations of the tortoises at the CDRS. In *G. n. hoodensis* follicular growth started to decrease in November immediately after the nesting season, and in *G. nigra* ssp., follicular growth had already started to decrease during the nesting season in September.

Geochelone gigantea, a giant tortoise species on Aldabra in the Indian Ocean (9°24'S, 46°20'E), also shows seasonal reproduction. Swingland and Coe (1978) have recorded similar sized follicles as the Galápagos tortoises, showing largest diameters (monthly means approximately 25–46 mm) between January and June, which coincides with the mating season and the beginning of the nesting season. Between July and November, largest follicular diameters in *G. gigantea* only ranges 5–10 mm (monthly means).

In *G. n. hoodensis*, smallest follicles measured had diameters of 36 mm (monthly mean). *G. nigra* ssp. showed an almost identical size of smallest vitellogenic follicles (monthly mean diameters of 33 mm). The difference between the sizes of the follicles of the Galápagos and the Aldabra tortoises might be related to food supply. The Galápagos tortoises at the CDRS feed on naturally growing plants within their corrals and additionally receive food three times a week, while the Aldabra tortoises suffer from a lack of forage due to overpopulation. Since there is little shade available on Aldabra atoll, many tortoises die from overheating and the competition for shade additionally forces the animals to stay underneath bushes instead of foraging (Swingland and Lessells, 1979). In turtles energy for vitellogenesis is either directly obtained from harvested resources or from stored lipids (Congdon and Gibbons, 1990). This might account for the smaller follicle sizes in *G. gigantea* since little food is available between July and November (cool and dry season) and energy supply for subsequent clutches may not yet be available.

Atretic follicles were observed throughout the year in the Galápagos tortoises at the CDRS, reaching maximal sizes of 62 mm in *G. n. hoodensis* and 56 mm in *G. nigra* ssp.. These exceptionally large atretic follicles have not been previously reported in chelonian species. Casares et al. (1997) and Robeck et al. (1990) reported maximal atretic follicle diameters of 47 and 38 mm in *G. nigra*. The large size of the follicles of the Galápagos tortoises undergoing atresia may be due to accumulation of fluid. Swingland and Coe (1978) have observed atretic follicles in *G. gigantea* that were frequently paired with developing preovulatory follicles and suggested that these atretic follicles may aid the energetic cost of yolk transport.

In females at the CDRS no post-ovulatory follicles (corpora lutea) could be identified during any of the ultrasound examinations. Using laparoscopy and ultrasound, Robeck et al. (1990) were also unable to distinguish older atretic follicles from old post-ovulatory follicles.

Throughout the year only a few complete copulations were observed in the tortoises of the CDRS. This low success ratio has also been reported in another group of *G. nigra* maintained in captivity (Casares et al., 1995). Different reasons could account for this. The animals could have been disturbed by the presence of the investigator, or complete copulations may have occurred at times of the day when the investigator was not present. Female turtles have been reported to store sperm over a long period of time (Gist and Jones, 1989; Gist et al., 1990; Galbraith, 1993; Gist and Fischer, 1993; Palmer et al., 1998), even as long as four years (Neubaum and Wolfner, 1999). It is likely that female Galápagos tortoises can also hold viable sperm for an extended period of time, which would allow them to be relatively independent of frequent mating successes.

The *G. n. hoodensis* tortoises at the CDRS showed oviductal periods ranging from 17 to 50 days. In captive Galápagos tortoises Casares et al. (1997) and Rostal et al. (1998b) reported oviductal periods of 34–84 and 18–76 days, respectively. These time periods seem to be extended compared with the oviductal period of the *G. n. hoodensis* tortoises but coincide with the findings in 4 protracted-nesting *G. nigra* ssp. females, where oviductal periods ranged from 8–74 days. However, it has to be considered that the female *G. nigra* ssp. live under suboptimal conditions. The differences in reproduction between the *G. nigra* ssp. and the *G. n. hoodensis* females can be explained by their habitat. Several times *G. nigra* ssp. females were observed seeking a nesting site on rocky soil for days, sometimes for weeks. The animals also dug an excessive number of incomplete nests before they finally laid their eggs. These stressful activities seem to be directly related to an inappropriate environment, forcing the animals to retain their eggs and to deposit them on the surface of the ground. The environmental circumstances could also account for the prolonged oviductal period in the "normally" nesting *G. nigra* ssp. females. Unlike the *G. nigra* ssp. tortoises, all *G. n. hoodensis* females always laid complete clutches and showed no evidence of retained eggs, which was validated by ultrasound examinations directly after the animals had laid their eggs.

The interclutch interval in the *G. n. hoodensis* females ranged from 30–64 days ($n = 8$). In the *G. nigra* ssp. females it ranged from 40–89 days ($n = 3$), excluding the tortoises which showed abnormal nesting behavior. Casares et al. (1997) described interclutch intervals in captive Galápagos tortoises at Zoo Zurich which exceeded several months or probably up to more than one year and were related to unusual long egg retention periods. Noegel and Moss (1989) also reported egg retention periods of more than one year in female Galápagos tortoises kept at the Life Fellowship Bird Sanctuary (Seffner, Florida). No other data regarding the interclutch interval in Galápagos tortoises are available.

The tortoises at the CDRS laid smaller clutches (*G. n. hoodensis*: 5–12 eggs; *G. nigra ssp.*: 5–14 eggs) than Galápagos tortoises kept in captivity. Clutch sizes in captive animals have ranged between 8–15 (Casares et al., 1997), 8–17 (Rostal et al., 1998b), 1–28 (Shaw, 1968), and 8–20 (Noegel and Moss, 1989). These differences between the tortoises on the Galápagos Islands and tortoises in captivity may be due to differences in diet. Clutch frequency varied between 1 and 3 nests per season in the *G. n. hoodensis* females and between 1 and 2 clutches per season in *G. nigra* ssp. In captive Galápagos tortoises Casares et al. (1997) reported 5 clutches from one animal during two nesting seasons. Rostal et al. (1998b) observed one female Galápagos tortoise nesting 4 times and another female nesting 2 times in one season. Noegel and Moss (1989) describe 3–5 clutches per season. Again, the higher number of clutches in captive animals may be related to diet. Although the Galápagos tortoises from Española (*G. n. hoodensis*) are approximately 100 years old (CDRS: L. Cayot, *pers. comm.*) they still reproduce successfully. Out of 145 eggs laid during the study period, 57.2% hatched (CDRS: H. Snell, *pers. comm.*).

In most female turtles follicular growth commences after the breeding season, which is described as a postnuptial cycle (Altland, 1951; Ernst, 1971; White and Murphy, 1973; Iverson, 1980; McPherson and Marion, 1981; Mitchell, 1985; Parmenter, 1985). Only a few tropical and subtropical turtle species display a prenuptial ovarian cycle, in which follicular development takes place just prior to mating (Singh, 1977; Wibbels et al., 1990; Rostal et al., 1997). The follicles of Galápagos tortoises start to grow at the onset of the mating season in January and reach their largest sizes at the end of the mating and during the nesting season. In turtles, high estradiol levels are an indicator of vitellogenesis (Lance and Callard, 1978; Gapp et al., 1979; Licht, 1982; Ho, 1987; Heck et al., 1997; Rostal et al., 1998a). Since estradiol levels in female Galápagos tortoises at the CDRS commence to increase before the onset of the mating season (Schramm et al., 1999a), we assume that females display a prenuptial ovarian cycle. This was validated by ultrasonography, showing follicular development prior to and at the beginning of the mating season.

Environmental factors such as temperature, precipitation, and light seem to play an important role in regulating the sexual cycles of male and female reptiles (Licht, 1972; Crews and Garrick, 1980; Licht, 1984; Whittier and Crews, 1987). In female turtles temperature may influence the reproductive potential (Moll and Moll, 1990) and the length of the reproductive season and clutch size (Mendonça, 1987). It may also affect the initiation of nesting (Gibbons and Greene, 1990) and the incubation period (Bobyn and Brooks, 1994) and may trigger the mating season (Kuchling and Dejose, 1989). Ganzhorn and Licht (1983) suggested that cooler temperatures in an aquatic, temperate zone turtle may stimulate vitellogenesis and ovulation. Both temperature and seasonal food availability probably regulate the seasonal reproductive cycle in the Galápagos tortoise.

In the tropics rainfall is a dominant environmental factor in regulating seasonality in reptiles (Licht, 1984). The Galápagos Islands lie on or close to the Equator and have a relatively constant day and night length. Santa Cruz Island (0°30'S, 90°10'W – 0°45'S, 90°30'W), where the study site was located, shows a photoperiod varying approximately ±

30 min throughout the year. Santa Cruz Island has a maximum elevation of 870 m and receives much higher precipitation in the highlands than Hood Island, which has a maximum elevation of 220 m and receives very little rainfall. However, the CDRS is situated at sea level and rainfall is somewhat similar to that of Hood Island (Jackson, 1993). Females at the CDRS mate during the hot and rainy season. Nesting occurs during the cool and dry period known as the *garúa* season when light drizzling rain is frequent. Captive Galápagos tortoises at Zoo Zurich nest between October and February when the animals are kept in a heated enclosure (Casares et al., 1997). In these animals regular mating attempts were observed throughout the year and courtship peaked between August and September (Casares et al., 1995). The Galápagos tortoises at Gladys Porter Zoo in Texas are housed in outdoor enclosures nearly year-round (Rostal et al., 1998b). Only during winter (December–February) do they spend some time in a heated barn. The animals breed from August to November and nest from November to April. Both of these zoo tortoise populations have shifted their nesting season by a couple of months. Since these tortoises were translocated to the northern hemisphere many years ago, the different climatic seasonality may account for this reproductive pattern.

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LITERATURE CITED

ALTLAND, P.D. 1951. Observations on the structure of the reproductive organs of the box turtle. J. Morph. 89:599-621.

BAKER, M.L. AND DALRYMPLE, G.V. 1978. Biological effects of diagnostic ultrasound: a review. Radiology 126:479-483.

BOBYN, M.L. AND BROOKS, R.J. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). J. Zool., Lond. 233:233-257.

CASARES, M. 1995. Untersuchungen zum Fortpflanzungsgeschehen bei Riesenlandschildkröten (*Geochelone elephantopus* und *G. gigantea*) und Landschildkröten (*Testudo graeca* und *T. hermanni*) anhand von Ultraschalldiagnostik und Steroidanalysen im Kot. Zool. Garten N.F. 65:50-76.

CASARES, M., HONEGGER, R.E., AND RÜBEL, A. 1995. Management of giant tortoises *Geochelone elephantopus* and *Geochelone gigantea* at Zurich Zoological Gardens. Int. Zoo Yb. 34:135-143.

CASARES, M., RÜBEL, A., AND HONEGGER, R.E. 1997. Observations on the female reproductive cycle of captive giant tortoises (*Geochelone* spp.) using ultrasound scanning. J. Zoo Wildl. Med. 28:267-273.

CAYOT, L.J. AND MORILLO, G.E. 1997. Rearing and repatriation of Galápagos tortoises: *Geochelone nigra hoodensis*, a case study. In: Van Abbema, J. (Ed.). Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference, New York: New York Turtle and Tortoise Society. pp. 178-183.

CONGDON, J.D. AND GIBBONS, J.W. 1990. Turtle eggs: their ecology and evolution. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Institution Press, pp. 109-123.

CREWS, D. AND GARRICK, L.D. 1980. Methods of inducing reproduction in captive reptiles. In: Murphy, J.B. and Collins, T. (Eds.). Reproductive Biology and Diseases of Captive Reptiles. Society for the Study of Amphibians and Reptiles 1:49-70.

ERNST, C.H. 1971. Sexual cycles and maturity of the turtle, *Chrysemys picta*. Biol. Bull. 140:191-200.

GALBRAITH, D.A. 1993. Review: multiple paternity and sperm storage in turtles. Herpetological Journal 3:117-123.

GANZHORN, D. AND LICHT, P. 1983. Regulation of seasonal gonadal cycles by temperature in the painted turtle, *Chrysemys picta*. Copeia 1983:347-358.

GAPP, D.A., HO, S.M., AND CALLARD, I.P. 1979. Plasma levels of vitellogenin in *Chrysemys picta* during the annual gonadal cycle: measurement by specific radioimmunoassay. Endocrinology 104:784-790.

GIBBONS, J.W. AND GREENE, J.L. 1990. Reproduction in the slider and other species of turtles. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Institution Press, pp. 124-134.

GIST, D.H. AND FISCHER, E.N. 1993. Fine structure of the sperm storage tubules in the box turtle oviduct. J. Reprod. Fert. 97:463-468.

GIST, D.H. AND JONES, J.M. 1989. Sperm storage within the oviduct of turtles. J. Morph. 199:379-384.

GIST, D.H., MICHAELSON, J.A., AND JONES, J.M. 1990. Autumn mating in the painted turtle *Chrysemys picta*. Herpetologica 46:331-336.

HECK, J., MCKENZIE, D.S., ROSTAL, D., AND MEDLER, K. 1997. Estrogen induction of plasma vitellogenin in the Kemp's ridley sea turtle (*Lepidochelys kempi*). Gen. Comp. Endocrinol. 107:280-288.

HO, S.M. 1987. Endocrinology of vitellogenesis. In: Norris, D.O. and Jones, R.E. (Eds.). Hormones and Reproduction in Fishes, Amphibians, and Reptiles. New York: Plenum Press, pp. 145-169.

IVERSON, J.B. 1980. The reproductive biology of *Gopherus polyphemus* (Chelonia: Testudinidae). Amer. Midl. Nat. 103:353-359.

JACKSON, M.H. 1993. Galapagos - A Natural History. University of Calgary Press, 315 pp.

KUCHLING, G. 1989. Assessment of ovarian follicles and oviductal eggs by ultrasound scanning in live freshwater turtles, *Chelodina oblonga*. Herpetologica 45:89-94.

KUCHLING, G. 1998. How to minimize risk and optimize information gain in assessing reproductive condition and fecundity of live female chelonians. Chelonian Conservation and Biology 3:118-123.

KUCHLING, G. AND BRADSHAW, S.D. 1993. Ovarian cycle and egg production of the western swamp tortoise *Pseudemydura umbrina* (Testudines: Chelidae) in the wild and in captivity. J. Zool., Lond. 229:405-419.

KUCHLING, G. AND DEJOSE, J.P. 1989. A captive breeding operation to rescue the critically endangered Western swamp turtle *Pseudemydura umbrina* from extinction. Int. Zoo Yb. 28:103-109.

LANCE, V. AND CALLARD, I.P. 1978. Hormonal control of ovarian steroidogenesis in nonmammalian vertebrates. In: Jones, R.E. (Ed.). The Vertebrate Ovary. New York: Plenum Press, pp. 361-407.

LICHT, P. 1972. Environmental physiology of reptilian breeding cycles: role of temperature. Gen. Comp. Endocrinol. Suppl. 3:477-488.

LICHT, P. 1982. Endocrine patterns in the reproductive cycle of turtles. Herpetologica 38:51-61.

LICHT, P. 1984. Reptiles. In: Lamming, G.E. (Ed.). Marshall's Physiology of Reproduction. Vol. 1. Reproductive Cycles of Vertebrates. Edinburgh: Livingstone, pp. 206-282.

MACFARLAND, C.G., VILLA, J., AND TORO, B. 1974. The Galápagos giant tortoises (*Geochelone elephantopus*). Part II: Conservation methods. Biol. Conserv. 6:198-212.

MCPHERSON, R.J. AND MARION, K.R. 1981. The reproductive biology of female *Sternotherus odoratus* in an Alabama population. J. Herpetol. 15:389-396.

MENDONÇA, M.T. 1987. Photothermal effects on the ovarian cycle of the musk turtle, *Sternotherus odoratus*. Herpetologica 43:82-90.

MITCHELL, J.C. 1985. Female reproductive cycle and life history attributes in a Virginia population of stinkpot turtles, *Sternotherus odoratus*. Copeia 1985:941-949.

MOLL, D. AND MOLL, E.O. 1990. The slider turtle in the neotropics: adaptation of a temperate species to a tropical environment. In: Gibbons, J.W. (Ed.). Life History and Ecology of the Slider Turtle. Washington, DC: Smithsonian Institution Press, pp. 152-161.

NEUBAUM, D.M. AND WOLFNER, M.F. 1999. Wise, winsome, or weird? Mechanisms of sperm storage in female animals. Current Topics in Developmental Biology 41:67-90.

NOEGEL, R.P. AND MOSS, G.A. 1989. Breeding the Galapagos tortoise, *Geochelone elephantopus*, at the Life Fellowship Bird Sanctuary, Seffner. Int. Zoo Yb. 28:78-83.

PALMER, K.S., ROSTAL, D.C., GRUMBLES, J.S., AND MULVEY, M. 1998. Long-term sperm storage in the desert tortoise (*Gopherus agassizii*). Copeia 1998:702-705.

PARMENTER, C.J. 1985. Reproduction and survivorship of *Chelodina longicollis* (Testudinata: Chelidae). In: Grigg, G., Shine, R., and Ehmann, H. (Eds.). Biology of Australasian Frogs and Reptiles. Royal Zoological Society of New South Wales, pp. 53-61.

PENNINCK, D.G., STEWART, J.S., PAUL-MURPHY, J., AND PION, P. 1991. Ultrasonography of the Californian desert tortoise (*Xerobates agassizi*): Anatomy and application. Vet. Radiol. 32:112-116.

PRITCHARD, P.C.H. 1996. The Galápagos Tortoises: Nomenclatural and Survival Status. Chelonian Research Monographs 1, 85 pp.

ROBECK, T.R., ROSTAL, D.C., BURCHFIELD, P.M., OWENS, D.W., AND KRAEMER, D.C. 1990. Ultrasound imaging of reproductive organs and eggs in Galapagos tortoises, *Geochelone elephantopus* spp. Zoo Biol. 9:349-359.

ROSTAL, D.C., ROBECK, T.R., OWENS, D.W., AND KRAEMER, D.C. 1990. Ultrasound imaging of ovaries and eggs in Kemp's ridley sea turtles (*Lepidochelys kempi*). J. Zoo Wildl. Med. 21:27-35.

ROSTAL, D.C., PALADINO, F.V., PATTERSON, R.M., AND SPOTILA, J.R. 1996. Reproductive physiology of nesting leatherback turtles (*Dermochelys choriacea*) at Las Baulas National Park, Costa Rica. Chelonian Conservation and Biology 2:230-236.

ROSTAL, D.C., GRUMBLES, J.S., BYLES, R.A., MÁRQUEZ-M., R., AND OWENS, D.W. 1997. Nesting physiology of Kemp's ridley sea turtles, *Lepidochelys kempi*, at Rancho Nuevo, Tamaulipas, Mexico, with observations on population estimates. Chelonian Conservation and Biology 2:538-547.

ROSTAL, D.C., OWENS, D.W., GRUMBLES, J.S., MACKENZIE, D.S., AND AMOSS, M.S. 1998a. Seasonal reproductive cycle of the Kemp's ridley sea turtle (*Lepidochelys kempi*). Gen. Comp. Endocrinol. 109:232-243.

ROSTAL, D.C., ROBECK, T.R., GRUMBLES, J.S., BURCHFIELD, P.M., AND OWENS, D.W. 1998b. Seasonal reproductive cycle of the Galapagos tortoise (*Geochelone nigra*) in captivity. Zoo Biology 17:505-517.

SCHILDGER, B.J., CASARES, M., KRAMER, M., SPÖRLE, H., GERWING, M., RÜBEL, A., TENTIU, H., AND GÖBEL, T. 1994. Techniques of ultrasonography in lizards, snakes, and chelonians. Semin. Avian Exotic Pet Med. 3:147-155.

SCHRAMM, B.G., CASARES, M., AND LANCE, V.A. 1999a. Steroid levels and reproductive cycle of the Galápagos tortoise, *Geochelone nigra*, living under semi-natural conditions. Gen. Comp. Endocrinol. 14:108-120.

SCHRAMM, B.G., CASARES, M., AND LANCE, V.A. 1999b. Reproductive cycles of male and female giant tortoises (*Geochelone nigra*) on the Galápagos Islands by plasma steroid analysis and ultrasound scanning. Linnaeus Fund Research Report. Chelonian Conservation and Biology 3:523-528.

SHAW, C.E. 1968. The giant's outlook. Zoonooz 41(11):12-29.

SINGH, D.P. 1977. Annual sexual rhythm in relation to environmental factors in a tropical pond turtle, *Lissemys punctata granosa*. Herpetologica 33:190-194.

SWINGLAND, I.R. AND COE, M.J. 1978. The natural regulation of giant tortoise populations on Aldabra atoll: reproduction. J. Zool., Lond. 186:285-309.

SWINGLAND, I.R. AND LESSELLS, C.M. 1979. The natural regulation of giant tortoise populations on Aldabra atoll. Movement, polymorphism, reproductive success and mortality. J. Animal Ecology 48:639-654.

WHITE, J.B. AND MURPHY, G.G. 1973. The reproductive cycle and sexual dimorphism of the common snapping turtle, *Chelydra serpentina serpentina*. Herpetologica 29:240-246.

WHITTIER, J.M. AND CREWS, D. 1987. Seasonal reproduction: patterns and control. In: Norris, D.O. and Jones, R.E. (Eds.). Hormones and Reproduction in Fishes, Amphibians, and Reptiles. New York: Plenum Press, pp. 385-409.

WIBBELS, T., OWENS, D.W., LIMPUS, C.J., REED, P.C., AND AMOSS, M.S. 1990. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). Gen. Comp. Endocrinol. 79:154-164.

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