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Female reproductive phenology in a population of Hermann's tortoise *Testudo hermanni hermanni* in Corsica

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We used radiography over two breeding seasons to investigate female reproductive phenology in a Corsican population of Hermann's tortoise. Wild females were kept in semi-captivity in similar conditions to the wild. The main purpose of the study was to determine the length of the breeding season for each female, the laying period, and the approximate length of the inter-clutch interval and clutch retention time. Clutches were laid only in May and June, and in both years the monthly proportions were similar. In 1998 the length of the breeding season (mean±SE = 44.7±3.3 days) was greater than in 1999 (33.1±2.6 days), but we found no annual differences in the date of first and second clutches. There were no differences in the inter-clutch interval between individual females or between years. Clutch retention time varied among clutches of the same year, but not between years for the same clutch order. When considering the order in which the clutches were laid, we observed that the first clutches were concentrated in May, whereas the second and third clutches were concentrated in June. In both years, only the females that laid their first clutches in May were able to lay a second or even a third clutch in June. Bigger females tended to lay first clutches earlier in the breeding season than did the smaller ones. The differences observed in the length of the breeding season between years may be explained by the annual differences in the frequency of females that produced one, two or three clutches, since we found no annual differences in the date of first and second clutches, in the inter-clutch interval or in the clutch retention time (for the same order of clutches).

Key words: Chelonia, clutch interval, egg-laying phenology, France, reproduction

INTRODUCTION

Female reproductive cycles in chelonians may be studied by hormonal analysis or by physical changes in reproductive structures. For living chelonians the latter can be efficiently monitored by ultrasound (Kuchling & Bradshaw, 1993; Kuchling, 1998; Henen & Hofmeyr, 2003) and, at least in part, by radiographic techniques (Hinton et al., 1997). From the changes in reproductive structures Moll (1989) described four periods in the female reproductive cycle for chelonians inhabiting temperate areas: 1) follicular enlargement, 2) ovulation and intrauterine period, 3) nesting period and 4) latent period. Hermann's tortoise *Testudo hermanni* may follow this annual cycle, but not all periods are well known. Their cycle begins with vitellogenesis in late summer, which is completed in the next spring (Casares et al., 1994), as in most chelonian species from temperate zones (Moll, 1989). Vitellogenesis is followed by the ovulation and intrauterine period in spring (Cruce & Raducan, 1976). Then comes the nesting period, mainly between May and June, which is the most studied period (Cruce & Raducan, 1976; Swingland & Stubbs, 1985; Hailey & Loumbourdis, 1988; Fertard, 1992; revision in Cheylan, 2001). No information, to our knowledge, has been published for the latent period of this species. To complete information on the reproductive cycle of the female Hermann's tortoise we studied female breeding phenology during the ovulation and nesting periods using radiography. The aims of the present study were to determine: 1) the length of the breeding season for individual females (ovulation of the first clutch to oviposition of the last); 2) the weekly frequency of gravid females during the breeding season; 3) the timing of the nesting period; 4) the approximate length of the inter-clutch interval (the number of days between two consecutive ovipositions); and 5) the approximate length of clutch retention time (the number of days from ovulation to oviposition). Other reproductive characteristics of this species are detailed elsewhere (Bertolero et al., 2007).

MATERIALS AND METHODS

Breeding phenology was studied using X-ray radiography (Hinton et al., 1997) on wild Hermann's tortoise females from the Porto-Vecchio population (Corsica, France, 41º36'N, 09º16'E), which were kept in semi-captivity for this work. In 1998, 20 females were available and in 1999, 12 more females were added to this first group. Each year during the nesting season (from April to mid-July) each female stayed in an individual outdoor enclosure (7–16m^2), with abundant natural vegetation (mainly *Vicia* sp., *Tuberaria* sp., *Arisarum* sp., *Scabiosa* sp. and several composites). During the non-reproductive period between 1998 and 1999, females stayed together in a big enclosure with several males. No additional food or water was provided from April to June 1998, since we assumed that the natural vegetation would cover the nutritional needs of the tortoises. Furthermore, in this fashion, we maintained the tortoises as close as possible to natural conditions (for example, the tortoises only had water after rainfall). Due to the drier conditions during spring 1999 (Fig. 1), we provided supplemental fresh wild vegetation in June 1999 (only plant species that tortoises eat frequently in the wild; Nougarède, 1998). Females were

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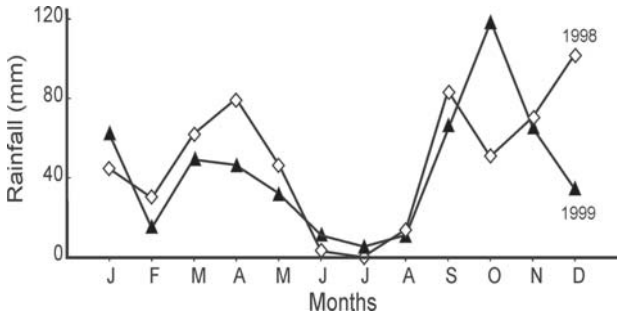

Fig. 1. Annual rainfall from the meteorological station of Chiappa (about 8 km from Porto-Vecchio, Corsica).

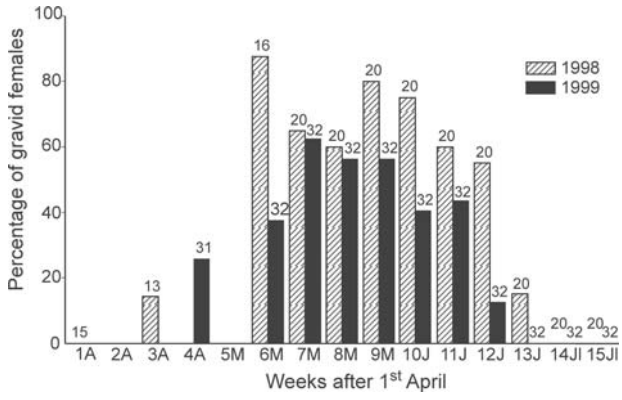

Fig. 2. Weekly frequencies of gravid Hermann's tortoise females in Porto-Vecchio (Corsica). The numbers above the bars indicate the number of females radiographed. The letters after each week's number indicate the month.

radiographed in 1998 between 4 April and 10 July (12 times; days between successive X-rays: mean±SE = 8.8±1.4), while in 1999 radiographs were taken between 28 April and 9 July (11 times; mean±SE = 7.2±0.5 days). The X-rays were taken by a veterinarian from a veterinary clinic in Porto-Vecchio. After 1999 all tortoises were released in the wild at their capture site.

The number of gravid females out of the total number of females X-rayed at each date is reported for each week from 1 April. To calculate the proportion of monthly gravid females each female was considered only once by month. We estimated approximate clutch retention time, inter-clutch interval and length of breeding season using the timing of the radiographs. The oviposition date was estimated as the mid-point between the date of the last X-ray showing the eggs of one clutch and the date of the following X-ray (mean±SE = 3.57±0.04 days, n=88). To determine clutch retention time, we calculated the difference between the oviposition date and the date of ovulation. The latter was estimated as the date halfway between the first X-ray in which a clutch was detected and the previous X-ray without eggs. We used 3.5 days in our calculations because it was half the time that elapsed between successive radiographs without and with clutches in May–July 1998 and in the 1999 breeding season (mean±SE = 3.54±0.03 days, n=60). Between 4 April and 9 May 1998, the interval between X-rays was too large to obtain a good estimate of the date of ovulation. Thus, for females whose first clutch was detected in two consecutive radiographs in April or in early May, we estimated that their clutches were ovulated 3.5 days before the date of the first X-ray showing eggs (following Henen et al., 2002). This procedure was not applied in the case of three females whose eggs were only detected in the first X-ray in May 1998, since the estimated time that the eggs were in the oviducts was extremely short (7 days) compared to other females (see results). The inter-clutch interval was defined as the number of days between two consecutive ovipositions. For each female, we defined the length of the breeding season as the time that elapsed between the estimated date of the ovulation of the first clutch and the estimated date of the oviposition of the last clutch. As an index of body size, we measured the straight carapace length (SCL) of each female as the midline distance from the anterior edge of the carapace to the posterior edge of the caudal scute with special callipers called a "tortometer" (Stubbs et al., 1994) to an accuracy of ±1 mm.

Because most data failed normality and homoscedasticity, the data were analysed with non-parametric tests following the indications of Sokal & Rohlf (1995) or of Quinn & Keough (2002). To compare the proportion of the clutches laid between month and years we used a G-test. We also used this test to compare the clutch frequency between years. We used Friedman's repeated measures ANOVA (with a post-hoc multiple pairwise testing procedure; Kutner et al., 2005), and Wilcoxon's test for matched pairs (T) to compare data for the same female between years or among clutches in the same year. To compare between clutches or years, we used the Mann–Whitney U-test (U). For correlations, we used Spearman's rank correlation (r_s). Analyses were carried out with STATISTICA 6.0. In some analyses where the same kind of data was available for the same female (several clutches for the same year or data for two years), we selected at random (by generating random numbers) data from only one of the clutches or one of the years (when this last selection at random was applied, it is de-

Table 1. The length of the breeding season and inter-clutch interval for Hermann's tortoise females in Porto-Vecchio (Corsica).

	Length of breeding season (days)			Inter-clutch interval (days)		
Years	Mean±SE	Median (range)	*n*	Mean±SE	Median (range)	*n*
1998	44.7±3.3	48 (20 – 69)	19	21.2±1.0	20.5 (17–27.5)	16
1999	33.1±2.6	35 (14 – 58)	26	18.9±0.9	21 (14–22.5)	16
Both years	-	-	-	20.2±0.8	20.6 (14–27)	20

Table 2. Laying dates and clutch retention time for Hermann's tortoises in Porto-Vecchio (Corsica). SE = standard error (number of days).

	Laying dates (day/month)			Clutch retention time (days)		
1998	Mean±SE	Median (range)	*n*	Mean±SE	Median (range)	*n*
1st clutch	23/05±2.4	16/05 (12/05–15/06)	19	18.7±1.2	20 (14–28)	16
2nd clutch	10/06±1.6	09/06 (02/06–22/06)	16	16.1±0.8	14 (13–21)	16
3rd clutch	24/06±1.2	22/06 (22/06–29/06)	9	12.9±0.7	13.5 (7–14)	9
1999						
1st clutch	28/05±2.2	31/05 (10/05–23/06)	26	20.5±0.6	21 (14–23)	26
2nd clutch	12/06±1.5	14/06 (31/05–23/06)	16	14.6±0.8	14 (7–21)	16
3rd clutch	23/06±0.0	23/06 (23/06)	2	15.5±0.0	15.5 (15.5)	2

noted "year at random"). The daily mean temperature for Corsica (1998–1999) was provided by the meteorological station in Chiappa, about 8 km from Porto-Vecchio. From this, the mean environmental temperature was calculated for each period of clutch retention of each female.

RESULTS

Females carried calcified oviductal eggs from April to June in both years (Fig. 2). No females radiographed in early April (04/04/98) and early July (03/07/98, 10/07/98, 02/07/99 and 09/07/99) showed any calcified oviductal eggs (Fig. 2). In both years May was the month with the greatest proportion of gravid females (1998: 95%; 1999: 71.9%), June being second (1998: 90%; 1999: 59.4%), whereas April was the month with the lowest proportion (1998: 13.3%; 1999: 25.8%). The breeding season was significantly longer in 1998 than in 1999 for females measured in both years (Wilcoxon's test for matched pairs, T=29, P=0.025, n=18) and for all the breeding females (Mann–Whitney U-test, U=154, P=0.033; Table 1). The frequency of clutches varied significantly among years, with more females that produced three clutches in 1998 than in 1999 (G=10.080, df=2, P=0.006). In both years, eggs were laid only in May and June (Fig. 3). The proportion of clutches laid every month was similar for both years (G_{adj}=2.228, df=1, P=0.136), but in 1998 egg-laying mainly occurred in June (63.6%) and in 1999 in May (52.3%; Fig. 3).

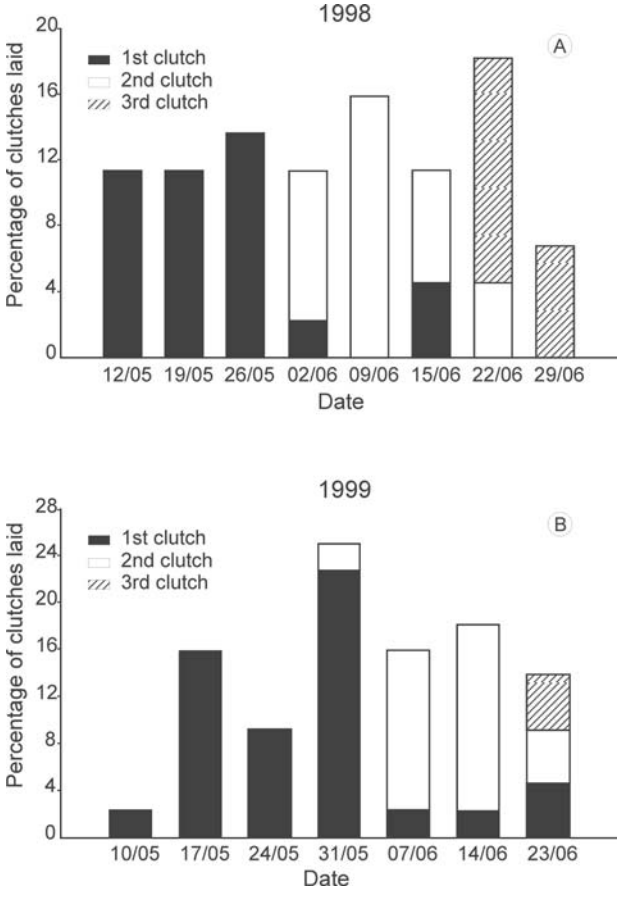

Fig. 3. The percentage of the total number of clutches laid at estimated laying dates for Hermann's tortoise females in Porto-Vecchio (Corsica): (A) 1998 and (B) 1999.

Despite the fact that in 1998 the first- and second-laid clutches tended to be earlier than in 1999 (Table 2), these differences were not significant for females that laid in both years (Wilcoxon's test for matched pairs, first clutch: T=84, P=0.948, n=18; second clutch: T=15, P=0.060, n=12). When considering all the females that laid in each year, we had the same results (Mann–Whitney U-test, first clutch: U=187, P=0.168; second clutch: U=123, P=0.851; Table 2). On the other hand, the number of clutches and the SCL were negatively correlated with the date of ovulation of the first clutch (Spearman's rank correlation, number of clutches: r_s=-0.434, P=0.024, n=27; SCL: r_s=-0.450, P=0.018, n=27; in both analyses, year at random). Thus, the females that oviposited earlier were bigger and had more clutches than the females that did so later.

We did not find significant differences in the inter-clutch interval of the same female (Wilcoxon's test for matched pairs, T=13, P=0.484, n=9; only 1998 with sufficient data), or between years (Mann–Whitney U-test, U=45, P=0.705, n=10 for both years; year at random, and mean values in the same year if the female laid three clutches; Table 1).

Clutch retention time varied among clutches from the same year (Friedman's repeat measures ANOVA, c^2=9.769, df=2, P=0.008; only 1998 with sufficient data; Table 2), but differences were only significant between the first clutch and the other two and not between the second clutch and the third one (testing limits for the three pairwise tests, clutches 1 and 2: 14.66–1.77, clutches 1and 3: 14.95–2.05, clutches 2 and 3: 6.73–-6.16). The clutch retention time did not vary between years for the

same clutch order (Wilcoxon's test for matched pairs, first clutch: T=18, P=0.182, n=15; second clutch: T=11, P=0.374, n=11). Thus, as the breeding season progressed, calcified eggs tended to remain for shorter periods in the oviducts (Table 2). On the other hand, clutch retention time was negatively correlated with the mean environmental temperature over the period (Spearman's rank correlation, r_S=-0.865, P<<0.001; choice at random of a single clutch per female and year). Thus, calcified eggs remained less time in the oviducts when the mean environmental temperature increased.

DISCUSSION

The period when females had calcified oviductal eggs (from mid-April to the end of June) coincides with the period of greatest activity in the annual cycle of female Hermann's tortoises (Huot-Daubrenot, 1996; Cheylan, 2001; Bertolero, 2002). We did not find gravid females in July, when temperatures are usually very high (the second warmest month of the year in Corsica; Kessler & Chambraud, 1986). The high temperatures from July onwards may be a factor limiting egg-laying in the summer, especially since Huot-Daubrenot (1996) found that females reach the greatest mean body temperature during egg-laying. In addition, Meek (1988) remarked that body temperatures increase very quickly when females nest.

Most clutches were laid between mid-May and the end of June (Fig. 3), thereby coinciding with most of the dates recorded in other parts of the distribution of Hermann's tortoise (Romania: Cruce & Raducan, 1976; South of France: Swingland & Stubbs, 1985; Fertard, 1992; Corsica: Nougarède, 1998; Greece: Swingland & Stubbs, 1985). Nevertheless, some of these studies also recorded oviposition before and after these dates: the first clutches were laid possibly as early as mid-April and the last clutches in early July (Swingland & Stubbs, 1985; Fertard, 1992; Nougarède, 1998; Cheylan, 2001). Thus, some flexibility in both the start and end dates of the nesting period exists, which may be related to the particular weather conditions in each locality in the year in question. On the other hand, most studies indicate that the peak egg-laying period occurs in June (Cruce & Raducan, 1976; Nougarède, 1998; Cheylan, 2001). Our results did not confirm these observations, since we found no difference in the proportion of clutches laid in May and June in each year.

When considering the order of the clutches, we observed that the first clutches were concentrated in May, whereas the second and third clutches were concentrated in June (Fig. 3). In both years, only the females that laid their first clutches in May were able to lay a second or even a third clutch in June. In addition, bigger females tended to lay first clutches earlier in the breeding season than did the smaller ones, but did not lay more clutches than the latter (Bertolero et al., 2007). Thus, it is possible that, in the case of the Hermann's tortoise, body reserves and habitat resources can limit clutch production, as was found for desert tortoises *Gopherus agassizii* (Henen, 1997; Wallis et al., 1999). On the other hand, the length of the breeding season does not seem to be the main factor that constrains clutch production, since, theoretically, a female would have enough time (inter-clutch interval) in June to lay a second clutch if she had already laid in that month. The differences observed in the length of the breeding season between years may be explained by the annual differences in the frequency of females that produced one, two or three clutches, since we did not find annual differences in the date of first and second clutches, in the inter-clutch interval or in the clutch retention time (for the same order of clutch).

For the Hermann's tortoise, Fertard (1992) found that the inter-clutch interval was 18.1±0.9 days, which is slightly lower than our results (20.2±0.8). This difference may be due to the fact that the method of determining inter-clutch interval was not the same in the two studies. For other populations of this species, records of inter-clutch interval are anecdotal, and extreme values reported are between 10 and 21 days (Cheylan, 2001).

Clutch retention time was influenced by the order of clutches as well as by the mean environmental temperature. Thus, as the environmental temperature increased through the nesting season, the clutches remained for shorter periods in the oviducts. These results are to be expected, since increases in temperature accelerate metabolism in reptiles (Bennett, 1982) and stimulate follicular growth (Duvall et al., 1982). Nevertheless, in order to obtain more precise estimates of the clutch retention time and the inter-clutch interval, it would be necessary to monitor females during the breeding season every two or three days. This would have to be done by ultrasound scanning, which would also allow detailed monitoring of egg development and reduce radiation risks to tortoises and their descendants (revision in Kuchling, 1998; Henen & Hofmeyr, 2003). But this kind of study may be very difficult in field conditions. Thus, studies carried out in semi-captive conditions with similar characteristics as in the wild may produce important results.

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