REPRODUCTIVE SEASONALITY AND CLUTCH SIZE OF SYMPATRIC HINGE-BACK TORTOISES (*KINIXYS EROSA* AND *KINIXYS HOMEANA*) IN SOUTHERN NIGERIA (REPTILIA, TESTUDINES: TESTUDINIDAE)

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ABSTRACT Tortoise specimens offered for food in local bush-meat markets were used to assess the reproductive seasonality and clutch size of sympatric *Kinixys erosa* and *K. homeana* from southern Nigeria (West Africa). Most females with oviductal eggs were found from November to March, with peaks in January-February (dry season). Very few females with oviductal eggs were found in the late wet season (August and September). For *K. homeana* at least, there was anatomical evidence of multiple clutches in a single year. However, regression statistics of rainfall against monthly frequency of occurrence of reproductive females demonstrate that females with oviductal eggs are associated to periods with low numbers of rain days per month. Females with eggs of *K. erosa* were significantly larger than those of *K. homeana*, and also produced a significantly higher number of eggs (x = 6.1 ± 1 eggs [N = 27] versus 5.4 ± 1.1 eggs [N = 41]). There was a significant positive relationship between female plastron length and number of oviductal eggs in either species, and the regression lines were not significantly different.

KEY WORDS Testudines, *Kinixys*, reproductive biology, ecology, Nigeria, tropical Africa

INTRODUCTION

A detailed knowledge of the reproductive biology of animals is crucial to design appropriate conservation plans (Burgman et al., 1993). Therefore, it is essential to get field data on the reproductive biology of a given vulnerable or threatened species before deciding any reliable management strategy for it.

The African forest hinge-back tortoises *Kinixys erosa* (Schweigger, 1812) and *K. homeana* Bell, 1827 have been subjected to a huge hunting pressure for both subsistence and traditional medicine (e.g. Butler & Shitu, 1985; Lawson, 2000, 2001; Luiselli et al., 2003), and are thus increasingly vulnerable in wide sectors of their range. At present, very few data is available on the reproductive biology of their free-ranging populations, apart from some general comments (Ernst & Barbour, 1989) or data coming from captive or ranched specimens (Harris, 2002). This is of course a serious shortcoming for conservation planning for these species.

In this paper we convey data on the reproductive seasonality and clutch size of sympatric *K. erosa* and *K. homeana* from southern Nigeria (West Africa). Compared to previous datasets (e.g. Harris, 2002), data reported here are of special note because they are entirely from free-ranging specimens. Another tortoise species, *K. belliana nogueyi* (Lataste, 1886), is found sympatric with the two above-mentioned species, but it is extremely rare at the study area (Luiselli et al., 2000, 2003; Luiselli, 2003a, 2003b). Therefore, *K. belliana nogueyi* is not considered in this study because we were unable to get a reliable sample size on it.

MATERIALS AND METHODS

Data were gathered from September 1996 to November 2002 in several localities in the eastern axis of the Niger Delta (states of Rivers, Bayelsa, Anambra, Akwa-Ibom, Abia, and Cross River), south-eastern Nigeria. The study region is tropical, with the wet season from May to October (Fig. 1). In this region, rainforest fragments (generally of the seasonal swampy type) are interspersed into a mosaic of farms, plantations, marshes, and urban centres. A detailed description of the study sites where most of the data were collected is presented in Luiselli et al. (2000).

Hinge-back tortoises are usually traded in local bush-meat markets (Butler & Shitu, 1985; Lawson, 2000; Luiselli et al., 2003). In order to find tortoises, we visited all the bush-meat markets situated along the courses of the rivers Sambreiro (= Sombreiro) and Orashi, and those in the vicinities of the following urban centres: Yenagoa, Sagbama, Port Harcourt, Peterside, Bonny, Obrikom, Oguta, Ahoada, Abonnema, Degema, Uyo, Eket, Calabar, Ugep, Akamkpa. Every possible effort was made to maintain a constant survey throughout the research period, i.e. on a monthly basis. Nevertheless, some minor sampling bias is likely, because it was impossible to maintain an identical monthly effort throughout the study period.

To collect natural history data, every traded tortoise was examined to species, sex, plastron length (with a calliper, along the midline of the plastron, to the nearest ± 1 mm), and dissected (if the specimen was already dead at the time of examination). The specimens that were still alive at the time of examination were bought, individually marked by notching a scale of the

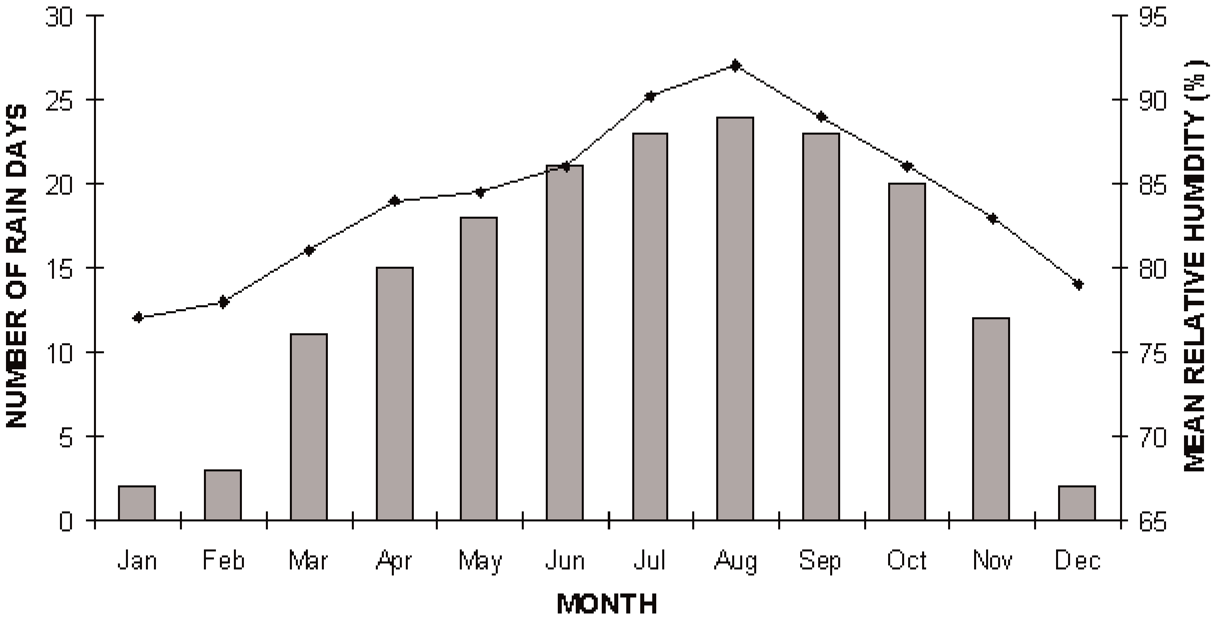

Fig. 1 Number of rain days per month (columns) and mean monthly relative humidity (line) at a typical locality of southern Nigeria where the two species of *Kinixys* are found sympatric (Calabar; data from the Department of Geography, University of Calabar).

carapace, and then set free in the nearest forest habitat where the presence of that species was known. As in other chelonian studies (e.g. Zuffi & Odetti, 1998), only those females with oviductal eggs were considered certainly reproductive. When dissection revealed the presence of oviductal eggs in a given female, the number of the eggs was noticed, as well as routine morphometric measures recorded for each tortoise.

Data from market surveys were compared with field data on free-ranging specimens that were marked and released during our staff's regular fieldwork. Free-ranging tortoises were routinely measured, and were permanently and individually marked by unique sequences of notches filed into the marginal scutes. Also in this case, gravid females were noted, although we were unable to count the number of their oviductal eggs by simple palpation of the inguinal region, as successfully done with other chelonians (Zuffi & Odetti, 1998). Indeed, as adult *K. erosa* have a projecting plastral lip at posterior end (Akani et al., unpublished data), the plastron of that species is proportionally longer compared to the shell length, thus causing some additional problems to counting eggs in the inguinal region.

All data were statistically analysed by STATISTICA (version 6.4, for Windows), with all tests being two-tailed and alpha set at 5%. Means are presented ± 1 SD.

RESULTS

Reproductive seasonality

Notwithstanding that the abundance of both species in bush-meat markets peaked during the wet season months as a consequence of the above-ground activity peaks of these species (cf. Lawson, 2000; Luiselli, 2003a, 2003b; Luiselli et al., 2003), most of the females with oviductal eggs were found in bush-meat markets during the dry season months, i.e. from November to March, with a peak in January-February (Fig. 2). Very few females with oviductal eggs were furthermore found in the late wet season (August and September, Fig. 2). The monthly frequency of occurrence of reproductive females was generally similar between species ($\chi^2 = 6.639$, df = 11, $P > 0.7$), therefore indicating high interspecific similarity in terms of reproductive seasonality. Assuming that the meteorological data for Calabar (Fig. 1) are reasonably representative of the meteorological conditions of the whole study region, and relating the number of rain days per month for this area to the monthly frequency of occurrence of reproductive females, these two variables were found to be negatively highly correlated in both *K. homeana* ($r = -0.849$, N = 12, $P < 0.00001$; equation: reproductive females monthly frequency = 1.656 - 1.146 × monthly number of rain days) and *K. erosa* ($r = -0.883$, N = 12, $P < 0.00001$; equation: reproductive females monthly frequency = 1.754 - 1.208 × monthly number of rain days). Females with oviductal eggs were therefore associated with periods

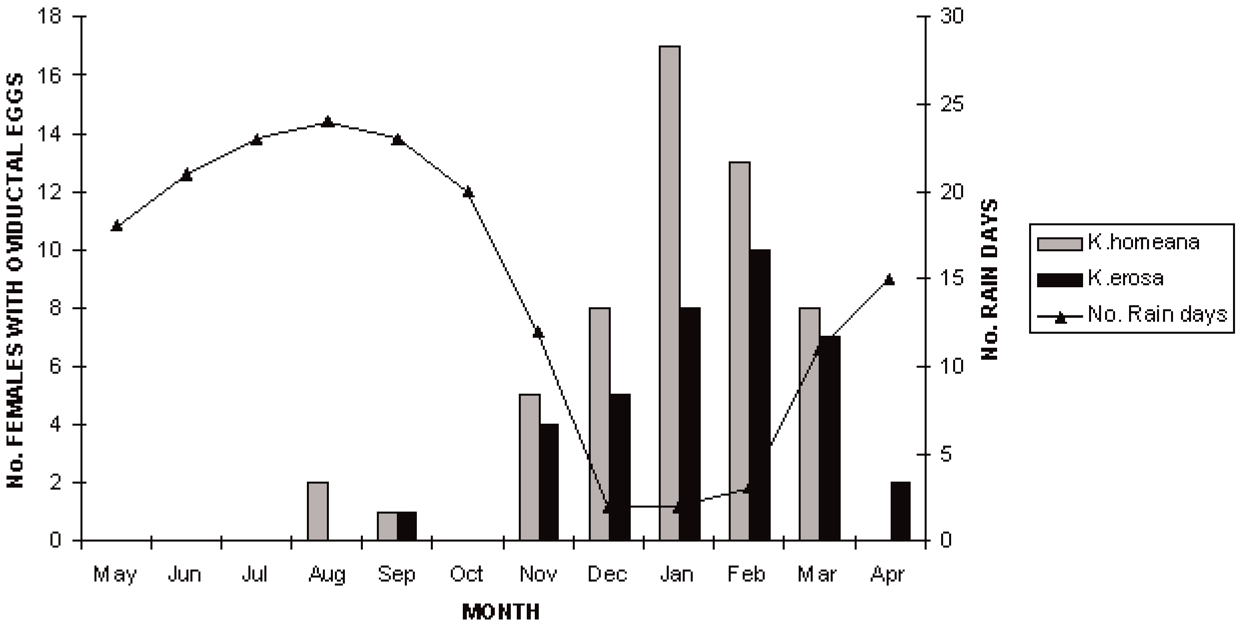

Fig. 2 Monthly numbers of female tortoises containing oviductal eggs in southern Nigeria, in relation to the number of rain days per month. All data came from dissection of bush-meat market specimens. Note that the peaks of females with oviductal eggs largely coincided with the dry months.

of low numbers of rain days per month, i.e. the dry season.

Clutch size

Oviductal eggs were recorded in 41 *K. homeana* and 27 *K. erosa*. The mean plastron length in the sample of *erosa* was significantly greater than in that of *homeana* (one-way ANOVA: $F_{1,66} = 9.106$, $P = 0.0036$).

Most of the females had 5-8 and 4-7 oviductal eggs in respectively *K. erosa* ($x = 6.1 \pm 1$ eggs, N = 27) and *K. homeana* ($x = 5.4 \pm 1.1$ eggs, N = 41), and means differed significantly between species (one-way ANOVA: $F_{1,66} = 6.645$, $P = 0.0121$). Female size (plastron length) and number of oviductal eggs in both *K. erosa* ($r = 0.872$, $F_{1,25} = 77.39$, $P < 0.0001$; equation: number of eggs = -19.92 + 0.126 × plastron length) and *K. homeana* ($r = 0.844$, $F_{1,39} = 96.77$, $P < 0.0001$; equation: number of eggs = -20.11 + 0.127 × plastron length) were significantly correlated, the slopes of these regression lines very similar between species (ANCOVA on slopes: $F_{1,63} = 0.007$, $P = 0.979$), indicating that the number of oviductal eggs increases with maternal plastron length in both species. This suggests that the higher mean fecundity of *K. erosa* depended merely on larger body size (plastron length).

DISCUSSION

Reproductive seasonality

Data on reproductive seasonality of Nigerian hinge-back tortoises are scarce. Mating season of Nigerian populations is reported to occur during the late phase of dry season (April; cf. Ernst & Barbour, 1989), but it should occur over a prolonged season, i.e. from March to November in both species in Togo (Harris, 2002). Lawson (2000) noted that *K. erosa* were frequently gravid during December in south-western Cameroon, and suggested that tortoises taken for bush-meat markets in this phase of the dry season may correspond to increased breeding and nesting activity at this time. Egg-laying should occur from January to April, and hatching of eggs in both species occurs in July in Togo (Harris, 2002). However, in contrast to data in this work and in Lawson (2000), all data from Togo came from captive or ranched specimens (Harris, 2002), and so may not be fully indicative of the reproductive seasonality of these free-ranging conspecifics. Data from this study (females with oviductal eggs during the dry season) showed that reproductive seasonality of both species in a climatically and environmentally homogenous region such as in southern Nigeria is better synchronized than might be expected on the basis of information from captive and ranched specimens in Togo.

The occurrence of a few specimens of either species with oviductal eggs in August-September probably indicates that fewer females in the population have a second oviposition phase (a well known phenomenon in chelonians, cf. Zuffi & Odetti, 1998). An alternative hypothesis is that there is some kind of sperm storage in at least a proportion of the reproductive females. As far as recording whether there are two or more clutches, the best way with an animal is to look for corpora lutea (oviductal scars left by follicles) in the ovaries. The number should be the same as the number of eggs, as each represents an ovulated follicle. If there are more, and the corpora lutea are in sets of two different sizes, then two clutches have been laid. In this work, ovarian condition was not checked accurately in the case of one wet season reproductive *K. erosa* and one *K. homeana*, but, in two other cases of *K. homeana* found with eggs in the wet season, the condition of the corpora lutea demonstrated that multiple clutches may occur in at least this species.

Clutch size

Other studies on chelonian reproduction have generally recorded the positive linear relationship between maternal size and number of eggs produced (e.g. Dodd, 1997; Tucker & Moll, 1997). Otherwise, this trend is also apparent in the great majority of extant species of reptiles studied up to now (cf. Shine & Greer, 1991 for a discussion of the cases of invariant clutch sizes). This pattern, generally known as 'Darwin's fecundity advantage model' (Shine, 1988), is therefore not surprising in *Kinixys* tortoises.

As for Nigerian populations of hinge-back tortoises, no data are available in the literature. Numbers of eggs per female were reported to be 2-6 in ranched specimens from Togo (Harris, 2002), and these data may be not fully indicative of the true clutch size of free-ranging conspecifics, since it is well documented that clutch size in reptiles can be influenced by food intake in captivity (Seigel et al., 2000; Ford, 2001; Seigel & Ford, 2001). Our data on both species indicated a higher mean clutch size for free-ranging *Kinixys*, and the differences from Harris' (2002) data can be attributed either to different body sizes between different populations, or unnatural conditions of captivity in Togo, or to different microclimatic conditions (Togolese populations live in somewhat drier external conditions than Nigerian ones), disfavouring the production of larger clutch sizes in Togolese populations. In this respect it must be noted that, while in Togo both *K. erosa* and *K. homeana* are found in just a few remnant forests, in Nigeria their distribution is much wider inside the rainforest and moist forest vegetation zone (Iverson, 1992; Luiselli et al., 2000). Unfortunately no body size data are reported for the Togolese sample studied by Harris (2002), because given that clutch size is strongly dependent on body size, we should need to know the size of Harris' tortoises before stressing that their smaller clutch sizes were due to captivity or microclimatic differences.

We also found that the two species of *Kinixys* were similar in terms of the relationship between maternal size and clutch size, although *K. erosa* produced a higher number of eggs, probably because of its larger body size (Ernst & Barbour, 1989). In this regard, it is questionable whether the interspecific difference in number of eggs is related to the difference in mean plastron length, because it would have been more useful to compare the mean masses of adult females of the two species, but unfortunately we did not collect weight data on a routine basis. It appears that interspecific differences in terms of clutch size observed in this study are lower than in Togo (cf. Harris, 2002), indicating that these tortoises may show different reproductive strategies in the various zones of their range in relation to external proximate conditions. To clarify this point, further comparative studies from other geographic areas would be essential.

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