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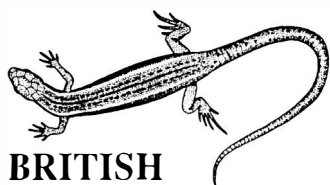
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FRONT COVER: *Chamaeleo chamaeleon* (P. Edgar)

REPRODUCTION OF *TROPIDURUS MONTANUS* RODRIGUES, 1987 (TROPIDURIDAE), A LIZARD FROM A SEASONAL HABITAT OF SOUTH-EASTERN BRAZIL, AND A COMPARISON WITH OTHER *TROPIDURUS* SPECIES

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The reproductive and fat body cycles of the lizard *Tropidurus montanus* (Tropiduridae) were studied in a seasonal, open habitat at Minas Gerais State, south-eastern Brazil. Lizards were sampled monthly from June 1996 to June 1997. Reproductive females were found from August to January. The smallest reproductive female was 56.5 mm in body size, and was collected in December. Mean clutch size (± 1 SD), based on counts of eggs or follicles, was 3.48 ± 1.15 , and was significantly related to female body size. Reproductive males were found throughout the year, but their frequency of occurrence varied greatly between months. The smallest reproductive male had a SVL of 60.6 mm, and was collected in August. For both sexes, fat-body mass (adjusted for body length) was greatest during the non-reproductive season.

Key words: lizards, reproduction, seasonality, south-eastern Brazil, *Tropidurus*

INTRODUCTION

A wide variety of reproductive strategies is known within lizards, and a major distinction between temperate and tropical species has been made concerning reproductive patterns (Tinkle *et al.*, 1970). According to this generalization, reproduction of temperate species is seasonal and mainly affected by temperature and photoperiod (Tinkle *et al.*, 1970; Licht, 1971), whereas in tropical lizards reproduction is considered to be aseasonal (Tinkle *et al.*, 1970; Benabib, 1994). However, such a broad generalization differentiating between temperate and tropical species no longer explains the diversity of reproductive patterns found in lizards. This is mainly because reproduction of many tropical species is now known to be related to seasonal changes in temperature, rainfall, humidity or ultraviolet light (Sexton *et al.*, 1971; Magnusson, 1987; Rocha, 1992; Clerke & Alford, 1993; Wiederhecker, 1999). Sherbrooke (1975) went a step further, and grouped the reproductive tactics of tropical lizards into three main categories: (1) continuous reproduction; (2) continuous with variation in reproductive activity; and (3) non-continuous. Vitt & Goldberg (1983) stated that a single reproductive pattern for tropical lizards was, thus far, not evident, and the actual determinants of seasonality in tropical lizards remain unclear (Vitt, 1992).

Evolutionary history has an overriding effect on most lizard life history traits, including reproduction, especially when considering higher taxonomic levels

(Dunham & Miles, 1985; Dunham *et al.*, 1988; Miles & Dunham, 1992). Vitt (1992) analysed the reproductive characteristics of lizards in different Brazilian sites and observed a diversity of reproductive strategies among species within single localities. He argued that if the environment was the main cause of variation, sympatric species would be expected to be more similar in their reproductive characteristics than different populations of the same species, whereas evolutionary history seemed to have a stronger influence. Nevertheless, while variation in reproductive patterns seems to be affected by evolutionary history, within some species of lizards variation exists that might be attributed to the local environment. For example, Colli (1991) and Vitt & Colli (1994) found that for the tropical lizard *Ameiva ameiva* (Teiidae) some life history characteristics differ among different populations, including the relationship between female body size, clutch size and the length of the reproductive season. These differences were mainly attributed to differences in predictability of rainfall, indicating that local environmental factors may also affect reproduction in tropical lizards.

Abdominal fat bodies are important lipid storage organs in lizards and exhibit a seasonal cycle in many species (Derickson, 1976). These cycles tend to be inversely related to reproductive cycles because lipid stored in fat bodies is used for reproduction (Derickson, 1976; Guillelte & Casas-Andreu, 1981; Vitt & Cooper, 1985). In females, fat bodies may be used during vitellogenesis, and in males they can be used for sperm production or courtship behaviour (Guillelte & Sullivan, 1985; Benabib, 1994). Although recognizing that the relationship between reproduction and fat bodies should also exist in tropical lizards, Magnusson (1987) proposed it would be weaker for tropical species than for temperate ones.

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The South American genus *Tropidurus* comprises mainly heliophilous, insectivorous lizards, living in open habitats, from semi-arid caatinga to seasonal cerrado, and to mesic lowland forests, where they occupy different microhabitats (Rodrigues, 1988; Vitt, 1993). Because these lizards have broad geographic ranges – and as many as four species may occur in sympatry or syntopy – they are ideal models for the study of variation in reproduction of tropical lizards. In general, when in seasonal habitats, these lizards have non-continuous reproduction with most reproduction occurring during the dry-wet season (*T. spinulosus* – Cruz *et al.*, 1997; *T. etheridgei* – Cruz, 1997; Cruz *et al.*, 1998; *T. itambere* – Van Sluys, 1993; *T. nanuzae* – Galdino, 2000; *T. torquatus* – Wiederhecker, 1999; *T. hispidus* – Prieto *et al.*, 1976), or dry season [*T.* (formerly *Platynotus*) *semitaeniatus* – Vitt & Goldberg, 1983]. Possible exceptions may be *T. torquatus*, at a coastal area in Espírito Santo State (south-eastern Brazil) where, despite seasonality in rainfall, females carrying oviductal eggs were found throughout the year (Teixeira & Giovanelli, 1999), and *T. hispidus* (formerly *T. torquatus*), at a seasonal habitat in north-eastern Brazil, which has an extended reproductive season lasting 11 months (Vitt & Goldberg, 1983).

Tropidurus montanus Rodrigues, 1987 is a medium-sized tropidurid endemic to the mountainous region of the Espinhaço mountain range, east of Minas Gerais State, south-eastern Brazil (Rodrigues, 1987). We studied the reproductive cycle of *T. montanus* in a seasonal habitat, specifically addressing the following questions: (1) do male and female *T. montanus* have seasonal or continuous reproduction; (2) to what extent is reproduction correlated with environmental variables such as temperature, rainfall and photoperiod; (3) what is the relationship between female body size and clutch size; and (4) how are fat body cycle and reproduction related? In addition, we compared the reproductive cycle of *T. montanus* with that of other *Tropidurus* species from different habitats.

MATERIAL AND METHODS

STUDY AREA AND CLIMATE

The study was carried out at a site in Serra do Cipó (19° 12'S/43° 27'W, 1260 m above sea level), at the southern portion of the Espinhaço mountain range, Minas Gerais state, eastern Brazil, near a stream called "Chapéu de Sol". The Espinhaço mountain chain ranges parallel to the eastern Brazilian coast, from 20°35'S to 11°11'S. In general, this mountain chain is characterized by quartzitic and arenitic outcrops (Giulietti *et al.*, 1987). At Serra do Cipó, the plant cover is diverse, and above 1000 m there is a predominance of grassy and herbaceous species (mainly Gramineae, Compositae, Melastomataceae, Eriocaulaceae, and Velloziaceae – Giulietti *et al.*, 1987).

The climate is highly seasonal, with warm and wet summers and dry winters (Giulietti *et al.*, 1987). The

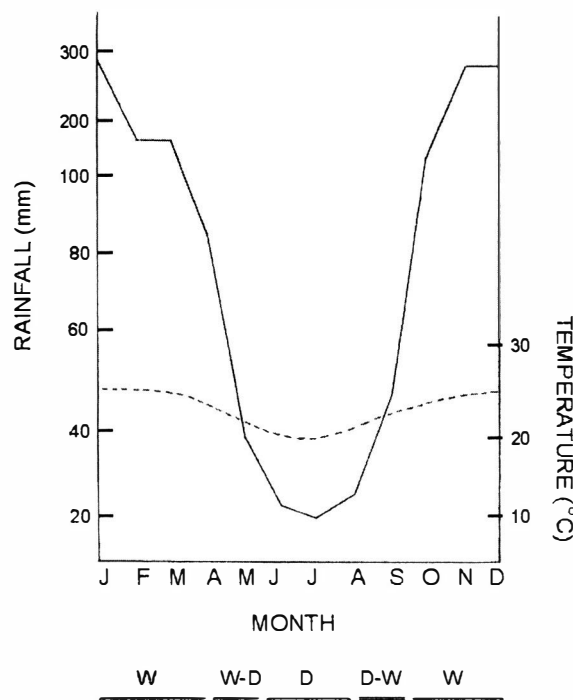


FIG. 1. Mean (1971-1990) monthly rainfall (in mm) and temperature (in °C) for Santana do Riacho, close to Serra do Cipó, Minas Gerais. The continuous line represents rainfall and the dashed line represents temperature. Bars below graph indicate the wet (W), dry (D) and transition (W-D and D-W) seasons.

dry season lasts from May to September and the wet season, from October to April (Fig. 1). Between 1971 and 1990 the mean annual rainfall in the area was 1540 mm and annual mean air temperature was 20.8 °C.

COLLECTING METHODS AND ANALYSIS

We collected lizards ($n=138$ females, 187 males) monthly from June 1996 to June 1997, using a noose or by hand. We measured each lizard's snout-vent length (SVL) to the nearest 0.1 mm, using a vernier caliper. We weighed each lizard with a Pesola spring scale (precision 0.2 or 0.5 g). The lizards were anaesthetized and killed in the field and were subsequently fixed in 10% formalin. Voucher specimens are deposited at the scientific collection of the Ecology Department, UERJ.

We assessed the reproductive status of each female lizard on the basis of vitellogenic follicles, oviductal eggs and/or corpora lutea. Follicles were considered vitellogenic when they were yellow and bigger than 3 mm in diameter (Van Sluys, 1993). We estimated clutch size, based on the number of vitellogenic follicles or oviductal eggs. The simultaneous presence of vitellogenic follicles and oviductal eggs was considered as evidence of the production of at least two clutches by that female in a single reproductive season. When there was any evidence of the production of more than one clutch, we considered only the number of oviductal eggs for the estimation of clutch size of that female. Relative clutch mass (RCM) was determined by dividing clutch

wet mass (preserved) by total mass (fresh clutch + body) (Vitt & Price, 1982).

For males, we measured the width and length of the testes to the nearest 0.1 mm using a digital caliper and estimated their volumes by using the formula for an ellipsoid, ($V = 4/3\pi ab^2$) (Mayhew, 1963; Van Sluys, 1993), where $a = 1/2$ of longest diameter and $b = 1/2$ of the smallest diameter. Left testis and the attached epididymes were removed, dehydrated, embedded in paraffin, sectioned at 5 μ m, mounted on slides, and stained with haematoxylin-eosin. Males were considered to be reproductive when they had spermatozoa in either the testes or epididymes. In addition, we measured the height of the germinal epithelium (HGE) and the diameter of the seminiferous tubules (DST) with an ocular micrometer to analyse reproductive activity in the testes. These measurements were taken in 10 different tubules and their means were used for statistical analysis.

Both abdominal fat bodies were removed and weighed on an electronic balance to the nearest 1 mg. The combined mass of fat bodies was log-transformed and subjected to linear regression on SVL; the residuals of this relationship were used as measures of fat body mass adjusted for lizard body size and were used in further statistical analysis.

The relationships between the proportions (arcsine transformed) of reproductive males and females and photoperiod, monthly rainfall and mean monthly temperature averaged over a 20-year period (1971-1990, long term rainfall – LT), and with a one-month time lag (short term rainfall – ST), were explored using linear regression analysis (Zar, 1999). The additive effect of these variables on reproduction was tested using multiple regression.

Testis measures (log-transformed testis volume, HGE and DST) were related to body size and, in the case of a significant relationship, the residuals were used in subsequent statistical analysis. In the figures we present all data, following Magnusson (2000).

RESULTS

Sampled males ranged in SVL from 30.5 to 98.0 mm, and females, from 39.0 to 82.6 mm. Adult males were significantly larger (mean \pm SD: 78.1 ± 10.51 mm, $n=129$) than adult females (69.79 ± 6.61 mm, $n=114$; $t=7.28$, $df=241$, $P<0.001$; Fig. 2).

We found reproductive females from August (39%) to January (94%; Fig. 3a). Females with vitellogenic follicles occurred from August to January, and oviductal eggs were found from October to January (Fig. 3a). In February 1997, two females out of eleven (18.2%) contained only corpora lutea; the others showed no evidence of reproductive activity. The smallest reproductive female was 56.5 mm in SVL, and was collected in December. We found four females (9.1%) with vitellogenic follicles and oviductal eggs, simultaneously, and they were collected in November and December, suggesting that at least two clutches may be produced by individual females during a reproductive

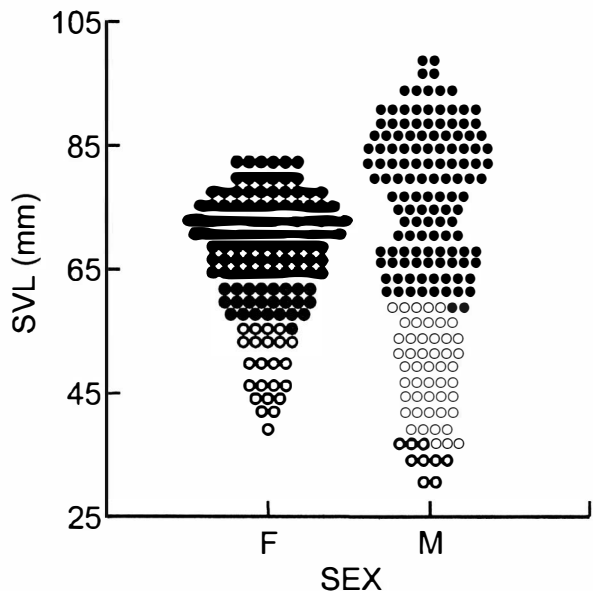


FIG. 2. Size distribution of male and female *Tropidurus montanus* at Serra do Cipó, Minas Gerais. Open circles represent juveniles and closed circles represent adults.

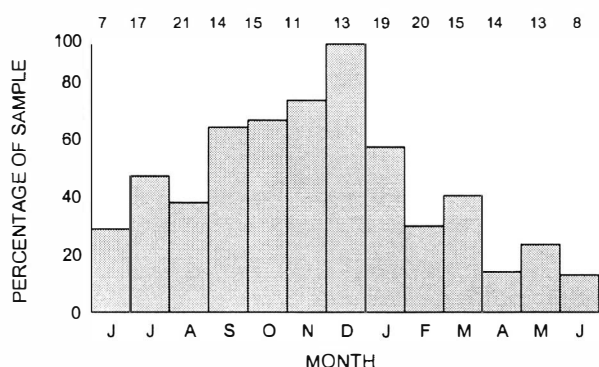
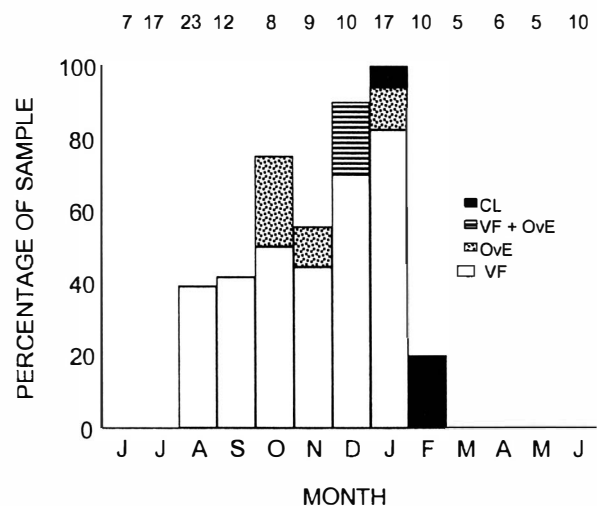


FIG. 3. Monthly percentages of reproductive *Tropidurus montanus* at Serra do Cipó, Minas Gerais, from June 1996 to June 1997: a) females and b) males. Numbers above each bar represent sample size. VF = vitellogenic follicles; OvE = oviductal eggs; CL = corpora lutea; VF + OvE = vitellogenic follicles and oviductal eggs.

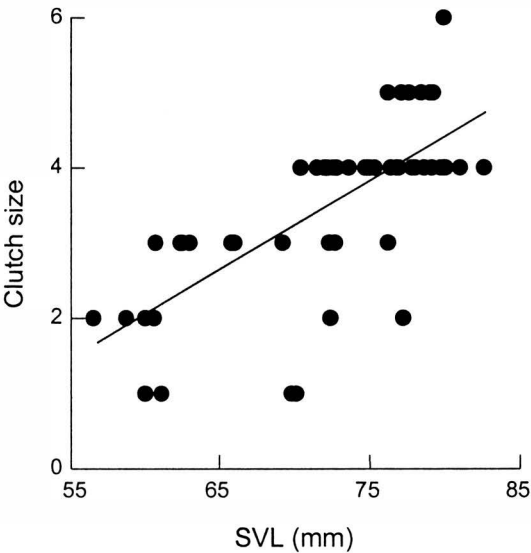


FIG. 4. Relationship between clutch size and female body size (SVL) of *Tropidurus montanus* at Serra do Cipó, Minas Gerais. Numbers beside graph represent sample size.

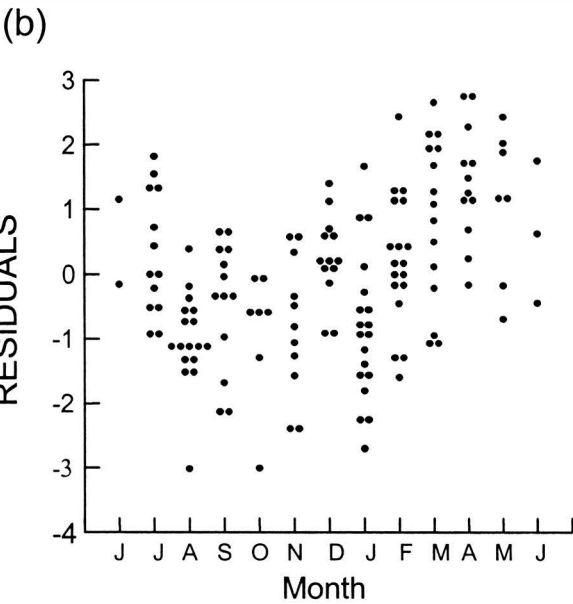
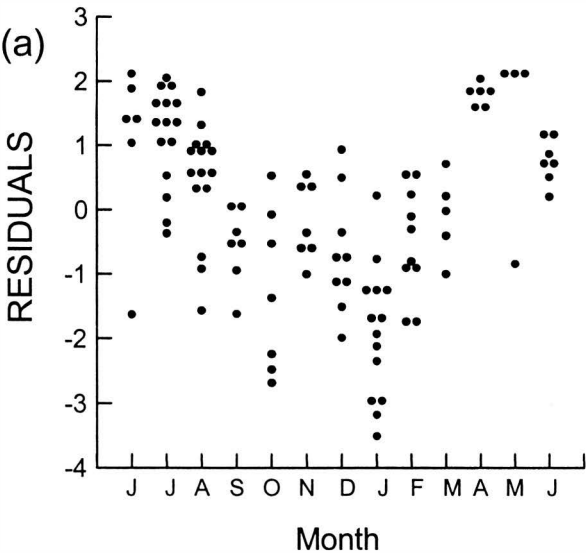


FIG 5. Monthly variation in size-adjusted testes volume in *Tropidurus montanus* at Serra do Cipó, Minas Gerais, from June 1996 to June 1997.

FIG. 6. Variation in size-adjusted abdominal fat-body mass of *Tropidurus montanus* at Serra do Cipó, Minas Gerais, from June 1996 to June 1997: (a) females; (b) males.

TABLE 1. Statistical results of the relationships between photoperiod, long term (LT) rain and temperature (1971-1990), and short term rain and temperature with one month time-lag (ST), and the frequency (arc-sin tranformed) of reproductive male and female *Tropidurus montanus* at Serra do Cipó, south-east Brazil.

Environmental variable	Males			Females		
	R^2	F	P	R^2	F	P
Photoperiod	0.53	12.571	0.005	0.54	12.988	0.004
LT rain	0.42	7.909	0.017	0.42	7.930	0.017
LT temperature	0.21	2.880	0.118	0.23	3.206	0.101
ST rain	0.12	1.351	.272	0.17	2.051	0.183
ST temperature	0.06	0.688	0.426	0.05	0.524	0.486

season. Mean \pm SD clutch size (based on counts of follicles or eggs) was 3.48 ± 1.15 (range: 1–6, $n = 52$), and was significantly related to female body size ($R^2 = 0.50$; $F_{1,50} = 50.2$; $P < 0.01$; Fig. 4). The mean relative clutch mass was $0.050 (\pm 0.043, n=20)$.

Reproductive males were found throughout the year, but the proportion varied among months (Fig. 3b). From September to January, the proportion of males in reproductive condition rose and from January the proportion declined. In December, 92.3% ($n=13$) of the males had spermatozoa in either the testis or epididymes (Fig. 3b). The smallest reproductive male had a SVL of 60.6 mm, and was collected in August. In February and April, four (11.8%) large adult males (SVL > 83 mm) had no spermatozoa in either the testis or epididymes.

Size-adjusted testis volume varied during the year, being largest between September and December (Fig. 5), the period with the highest proportion of reproductive males. Size-adjusted DST and HGE varied similarly and testis volume was significantly correlated with both measures (testis volume vs DST: $r=0.92$; testis volume vs HGE: $r=0.91$ and $P < 0.001$, for both correlations). After December, all values of these measures decreased considerably.

The monthly proportions of males and females in reproductive condition were significantly correlated with photoperiod and long-term monthly means of rainfall, but not with monthly means of temperature (Table 1).

For both males and females, size-adjusted fat-body mass varied similarly during the year. Fat bodies of both sexes increased in mass rapidly before the onset of the reproductive season and subsequently decreased during the reproductive season (Fig. 6). The variation in fat body mass appeared to be greater in females than in males.

DISCUSSION

Reproduction in *T. montanus* at Serra do Cipó is seasonal, starting during the middle of the dry season and lasting until the middle of the wet season (February). Females seem to have a more pronounced reproductive cycle than males, and from February to July we found no reproductive females. During every month some males were in reproductive condition (i.e. producing sperm). The proportion of sperm-producing males varied, being higher during the wet season, at the same time when there were reproductive females. The fact that some large adult (mature) lizards had no spermatozoa in either testis or epididymes in the months after the reproductive season (February and April) suggests they were post-reproductive and that there may be a period of time when individual males are reproductively quiescent. The exact significance of the presence of reproductive males during all months of the year is not clear, but may be due to high levels of sexual hormones in the blood. As suggested by Wiederhecker (1999) for *T. torquatus*, this may affect those aggressive behaviours that might be important for territory maintenance.

The time interval between the first female found with vitellogenic follicles (August) and the first female found with oviductal eggs (October) were found suggests that two months are required to produce a clutch.

At the study site, arthropod availability is highly seasonal, being rather greater during the wet season than during the dry season (Kiefer, 1998). Thus, when young lizards hatch, environmental conditions are propitious, with more food enabling them to grow quickly and reach a body size at which they are less vulnerable to predators (Vrcibradic & Rocha, 1998). In addition, because moisture is a critical factor for egg survival (Wiederhecker, 1999), females laying eggs during the wet season may enhance their reproductive success by reducing losses caused by desiccation.

In *T. montanus* fat-body mass increased during the dry season, and the greatest values were recorded during this time. Guillelte & Casas-Andreu (1981) stressed the importance of food availability on fat-body mass in tropical lizards, arguing that supplementary feeding increases fat body size. At our study site, Kiefer (1998) found some seasonal variation in arthropod availability, which was lower during the dry season (June to August), but the fat body cycles indicate there is enough food for adults to increase their lipid levels during the dry season. Our data suggest that the seasonal cycle in fat-body mass of *T. montanus* at Serra do Cipó is inversely related to the gonadal cycle of both males and females, as is known for many other lizard species (e.g. Derickson, 1976; Vitt & Cooper, 1985; Benabib, 1994; van Wyk, 1994; Van Sluys, 1998; Vrcibradic & Rocha, 1998). Vitellogenesis starts during the dry season, when food availability is lower, suggesting that females rely heavily on lipid stored in their abdominal fat bodies. However, as the proportion of females producing more than one clutch was low, it is not clear whether they rely on food for the production of this second clutch, as suggested by Benabib (1994) for *Sceloporus variabilis*, and Wiederhecker (1999) for *Tropidurus torquatus*. The lowest values of fat-body mass were observed during the wet season, when food availability is greatest (Kiefer, 1998), suggesting that lipid depletion was due to its utilization for gonad maturation and reproduction. The difference between male and female fat body cycles may reflect a higher cost of reproduction for females (e.g. Vrcibradic & Rocha, 1998 and included references). The dynamics of seasonal energy storage in males have only been reported for a few species, mostly showing that lipid storage in females is greater than in males (van Wyk, 1994). Yolk deposition during egg production is energetically expensive for female lizards (van Wyk, 1994).

Analysing the reproductive cycle of species in the genus *Tropidurus* for which there are data (16 species, 22 populations; Table 2), we observe a few general patterns. For those species that have been studied for a full year, the reproductive cycle is similar to that found for *T. montanus*, i.e. reproductive males were found

TABLE 2. Reproductive characteristics of *Tropidurus* species used for comparisons with *T. montanus* from Serra do Cipó, Minas Gerais; a, mean, range in parentheses; b, ?, not a year-round study.

Species	Locality	Clutch size ^a	Male reproductive cycle ^b	Female reproductive cycle ^b	Habitat	Source
<i>T. torquatus</i>	Brasília, CW Brazil	6.1 (3-10)	year round	6 months	cerrado	Wiederhecker (1999)
<i>T. torquatus</i>	Guriri, SE Brazil	- (2-4)	—	year round	restinga	Teixeira & Giovanelli (1999)
<i>T. itambere</i>	Valinhos, SE Brazil	3.5 (1-8)	year round	5 months	rock outcrops	Van Sluys (1993)
<i>T. montanus</i>	Serra do Cipó, SE Brazil	3.5 (1-6)	year round	6 months	rock outcrops	This study
<i>T. cf. montanus</i>	Alto do Araguaia, CW Brazil	?	?	?	cerrado	Vitt, (1991a)
<i>T. nanuzae</i>	Serra do Cipó, SE Brazil	2.1 (1-3)	year round	9 months	rock outcrops	Galdino (2000)
<i>T. semitaeniatus</i>	Exu, NE Brazil	2	year round	7 months	caatinga	Vitt & Goldberg (1983)
<i>T. hispidus</i>	Exu, NE Brazil	7.4 (3-14)	year round	year round	caatinga	Vitt & Goldberg (1983)
<i>T. hispidus</i>	Venezuela	5.6 (1-8)	7 months	6 months	thorn forest	Prieto <i>et al.</i> (1976)
<i>T. plica</i>	Amazonian Brazil	2.9 (1-5)	?	?	forest	Vitt (1991b)
<i>T. umbra</i>	Amazonian Brazil	1.9 (1-2)	?	?	forest	Vitt <i>et al.</i> (1997)
<i>T. flaviceps</i>	Ecuador	2	?	?	forest	Vitt & Zani (1996)
<i>T. spinulosus</i>	Sierra Grande, Argentina	5.7 (4-8)	year round	4 months	thorn forest	Martori & Aùn (1994)
<i>T. spinulosus</i>	Alto do Araguaia, CW Brazil	4.0 (3-6)	?	?	cerrado	Vitt (1991a)
<i>T. spinulosus</i>	Salta, Argentina	5.0 (3-7)	3 months	3 months	dry chaco	Cruz <i>et al.</i> (1997)
<i>T. etheridgei</i>	Salta, Argentina	6.2 (3-9)	5 months	5 months	dry chaco	Cruz (1997)
<i>T. etheridgei</i>	Alto do Araguaia, CW Brazil	4.9 (3-7)	?	?	cerrado	Vitt (1991a)
<i>T. etheridgei</i>	Salta, Argentina	5.9 (?-9)	5 months	6 months	dry chaco	Cruz <i>et al.</i> (1998)
<i>Tropidurus</i> sp1	Santa Barbara, N Brazil	3.4 (2-5)	?	?	rock outcrops	Vitt (1993)
<i>Tropidurus</i> sp2	Ariquemes, N Brazil	3.5 (2-4)	?	?	rock outcrops	Vitt (1993)
<i>Tropidurus</i> sp3	Santa Cruz, N Brazil	3.8 (1-5)	?	?	rock outcrops	Vitt (1993)
<i>T. cf. oreadicus</i>	Pará, N Brazil	4.2 (2-6)	?	?	rock outcrops	Vitt (1993)
<i>T. itambere</i>	Pirenópolis, CW Brazil	3.6 (1-8)	?	?	rock outcrops	Faria (2001)
<i>T. oreadicus</i>	Pirenópolis, CW Brazil	3.7 (1-6)	?	?	rock outcrops	Faria (2001)

throughout the year but their proportion varied; whereas reproductive females were found only during part of the year (Table 2). *Tropidurus hispidus* from a caatinga habitat (Exu, Pernambuco), and *T. torquatus* from a restinga area (Espírito Santo) are probably exceptions to that pattern, because reproductive individuals were found for most of the year. For most seasonal species, reproduction occurs from the middle of the dry season to the wet season. The similarity of the reproductive patterns found across species in different habitats, ranging from forests to open-habitats, suggests that there is a general pattern of seasonality in reproduction in *Tropidurus* lizards.

Considering the effect of local environmental variables on the reproductive cycle of *Tropidurus* lizards, only a few comments can be made because for only a few species is there any analysis evaluating the effect local environmental factors may have on reproduction. Only the studies of Wiederhecker (1999), Galdino (2000) and Van Sluys (1993) considered the effect of rainfall, temperature and photoperiod on reproduction of *T. torquatus*, *T. nanuzae* and *T. itambere*, respectively. In these species the reproductive cycle was significantly correlated with seasonality in rainfall and/or variation in photoperiod. However, experiments should be carried out to test whether these factors really cause reproductive seasonality, independent of phylogeny. At present, then, it is difficult to clearly understand the role environmental factors might have on reproduction of *Tropidurus* species.

Clutch size for *T. montanus* was significantly related to female body size, which explained about half of the variation in the former. A significant relationship between clutch size and female body size is a pattern in many open-habitat *Tropidurus* species (Vitt & Goldberg, 1983; Vitt, 1993; Van Sluys, 1993; Cruz, 1997; Cruz *et al.*, 1997; Cruz *et al.*, 1998; Wiederhecker, 1999). However, such a correlation has not been demonstrated for some species/populations (*T. semitaeniatus* – Vitt & Goldberg, 1983; *T. spinulosus* and *T. etheridgei* – Vitt, 1991a; two of the undescribed species from Ariquemes and Santa Cruz – Vitt, 1993; *T. flaviceps* – Vitt & Zani, 1996, and *T. nanuzae* – Galdino, 2000). Rand (1982) found that Amazonian lizards tended to have smaller clutches than similar species in open habitats. However, Vitt & Zani (1996) argue that there seems to be no clear pattern in clutch size between species of forested and open areas. When clutch size is affected by female body size, it may indicate that natural selection would favour larger females, because they would tend to produce larger clutches. However, females might also increase their fitness by laying more clutches during each reproductive season. A relatively constant clutch size and the consequent lack of an effect of female body size on clutch size may be advantageous for habitat-specialists such as the forest-dweller *Tropidurus flaviceps* (Vitt & Zani, 1996) and the crevice-dweller *T. semitaeniatus* (Vitt & Goldberg, 1983) or small-sized species (*T. nanuzae*, Galdino, 2000). Only

about 9% of the females that could potentially bear oviductal eggs (those found from October to January, $n=44$) showed signs of producing more than one clutch per reproductive season and this suggests that *T. montanus* females may enhance their reproductive success by growing fast and not by laying multiple clutches.

Relative clutch mass of *T. montanus* at our study site was similar to that of *T. torquatus* at a cerrado site (mean \pm SD: RCM 0.056 \pm 0.138; Wiederhecker, 1999), but smaller than that found for other *Tropidurus* species both in rainforest (0.11 for *T. flaviceps* – Vitt & Zani, 1996; 0.124 for *T. [formerly Plica] plica* – Vitt, 1991b and 0.154 for *T. umbra* – Vitt, Zani & Ávila-Pires, 1997), and in open areas (Vitt, 1993; Cruz *et al.*, 1997). The difference between *T. montanus* and the congener *T. nanuzae* in RCM (larger in *T. montanus*) and the relationship between clutch size and female body size (not significant for *T. nanuzae* – Galdino, 2000) may be due to the small body size of *T. nanuzae* (mean \pm SD female SVL = 51.6 \pm 4.1mm; Kiefer, 1998). Both species are saxicolous and use rock crevices as shelters. The reason for this difference is still open to testing.

Because of the general patterns found, irrespective of habitat, at this point it seems that reproduction in *Tropidurus* lizards is greatly affected by their evolutionary history. Nevertheless, more detailed studies on reproduction of *Tropidurus* species with broad geographic distributions (e.g. *T. torquatus*, *T. hispidus* and *T. itambere*), and sympatric species, might allow a test of the relative influence of phylogeny and local environmental conditions on reproduction of these lizards.

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REPRODUCTION OF *CHAMAELEO CHAMAELEON* UNDER CONTRASTING ENVIRONMENTAL CONDITIONS

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Reproductive characteristics of *Chamaeleo chamaeleon* are described and compared between a dry and a wet year. Nesting occurred in September and October, beginning later in the dry year when females also excavated their nests in longer (but not deeper) tunnels. Females were significantly smaller, with lower body mass, lower clutch mass and lower clutch size in the dry year. Relative clutch mass and body condition did not differ between the two years. Females laid a single clutch of 4-40 eggs, with a mean relative clutch mass of 60-70%. Clutch size was correlated with maternal size, being larger in the wet year, whereas egg variables were independent of maternal characteristics. Higher mortality rate was recorded in the dry than in the wet year. The observed variation in reproductive output may be explained as a consequence of lower availability of food resources in the dry year, resulting in lower fecundity and survival of females.

Key words: chameleons, clutch size, environmental influence, maternal condition

INTRODUCTION

In reptiles, reproductive activities are influenced by environmental factors, such as temperature, rainfall and food supply. The variation in these factors are frequently associated with variation in reproductive traits, revealing their plasticity as a function of available energy (Dunham, Miles & Reznick, 1988; Seigel & Ford, 1991). Intraspecific variation in clutch size and frequency (as a consequence of different environmental causes) has been reported for several species of reptiles (Vitt, van Loben Sels & Ohmart, 1978; Seigel & Ford, 1991 and references therein), and experimental studies have demonstrated that food availability may be the main ecological factor underlying differences among individual females (Seigel & Ford, 1991; Olsson & Shine, 1997). Individual variation in reproductive output can also be explained by physical constraints, mainly related to body size, as the female abdominal cavity determines an upper limit for reproductive investment (Vitt & Congdon, 1978; Shine, 1992).

The common chameleon, *Chamaeleo chamaeleon*, inhabits a wide area around the Mediterranean sea. It is an oviparous arboreal lizard with summer courtship, autumn oviposition and a protracted incubation time (Bons & Bons, 1960; Blasco *et al.*, 1985; Cuadrado & Loman, 1999), characteristics that differentiate chameleons from the general pattern of other Mediterranean reptiles. In a previous paper, we described the process of nest construction and reported the difficulties that some female chameleons exhibit during oviposition. Females dig long and deep tunnels for nesting, and under severe drought conditions, some of them were observed to become exhausted and eventually die (Blázquez, Díaz-Paniagua & Mateo, 2000).

In the present paper, we report reproductive characteristics of *Chamaeleo chamaeleon* in southern Spain and its variation in two climatically contrasting years (drought versus high rainfall). Our aim was to analyse the variation in female reproductive investment in this population, presumably as a function of environmental conditions; as it was not an experimental study, we did not pretend to establish certain correlations between reproduction and weather.

METHODS

The study was conducted in Rota and San Fernando, in Cádiz province (36° 28'N, 6° 12'W) in southern Spain, where chameleons inhabit sandy coastal areas closely associated with human activities. The study area included semi-abandoned farms and gardens, where vegetation mainly consisted of dispersed shrubs of *Retama monosperma*, and garden trees (*Myoporum tenuifolius*, *Prunus amygdalus*, *Punica granatum*, etc.). The climate is Mediterranean with hot, dry summers and mild winters, and rains fall mainly in autumn and winter.

Field work was carried out from September to November in 1995 and 1996. Climatic conditions differed remarkably between years (Fig. 1): 1995 was very dry (total rainfall November 1994-October 1995: 201mm), whereas 1996 was very wet (total rainfall November 1995-October 1996: 917 mm).

In both years we searched intensively for nesting or gravid females (which showed a voluminous clutch through the abdominal wall). Field work was concluded when no nesting females remained in the study area. Chameleons were captured by hand, and their snout-vent length (SVL) was measured to the nearest 0.5 mm. They were marked individually with spots of nail polish on the limbs (in 1995) and by claw-clipping (in 1996). Body mass was recorded to the nearest 0.5 g with a spring dynamometer before oviposition (BMB) and after oviposition (BMA), whenever possible. When both

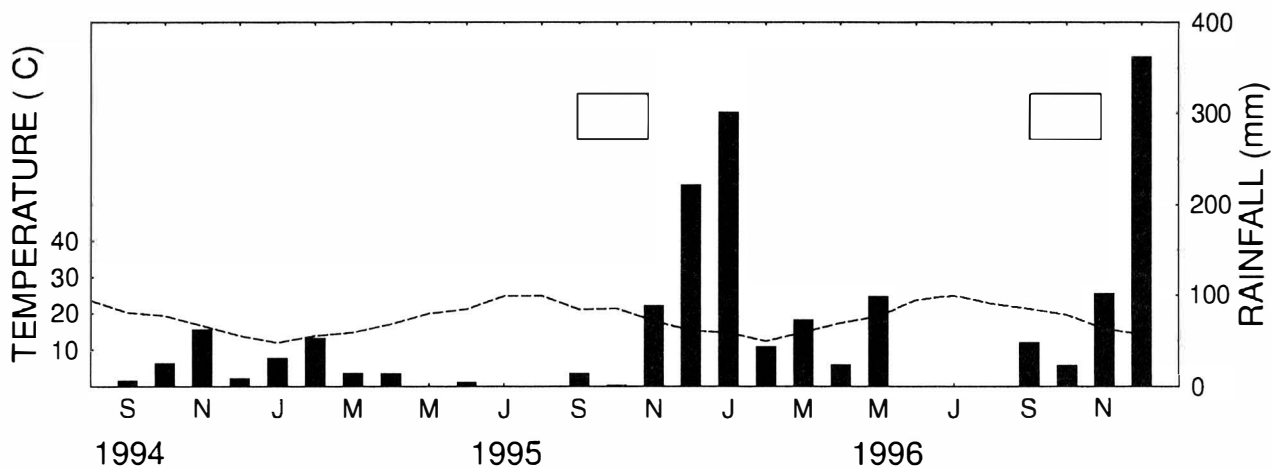


FIG. 1. Monthly variation in rainfall (bars) and mean temperature (broken line) during the period of study of reproduction by chameleons in southern Spain. Horizontal bars above the histogram indicate the nesting season in each study year.

values were recorded for an individual female, their difference (BMB-BMA) was considered as an approximation of the clutch mass (ACM). This measure is not an entirely satisfactory measure of clutch mass, but we considered it appropriate to describe the high increment in mass supported by females due to their reproductive investment. BMB was the maximum body mass of egg-bearing females, recorded on dates close to oviposition, whereas BMA was recorded from 0 to 2 days after nesting. Relative clutch mass (RCM) was estimated as the ratio ACM/BMA (following Shine, 1980).

Clutch size (CS) was recorded either by opening the nests immediately after egg-laying ($n=19$ in 1995), after eggs had hatched ($n=5$ in 1995, $n=24$ in 1996), and from dissection of gravid females found dead (five in 1995 and one in 1996). Egg length (EL), egg width (EW) and egg mass (EM) were recorded on the nesting day for 19 clutches in 1995. In 1996 we measured only four eggs in each of 35 clutches. Egg shape was calculated as the ratio of EL to EW.

Nests were located when we observed females excavating on the ground, and by tracks left on the sand by females after nesting. The depth (vertical distance from the ground surface to the egg chamber) and length of the nest tunnel were measured in 14 nests in 1995 and 23 in 1996.

Mean values per clutch were used in all statistical analyses. Variables were compared among females and between the two study years using ANOVA and Pearson correlation. Correlations between length variables (SVL, EL, EW) and mass variables (BMA, BMB, ACM, EM) were made after their logarithmic transformation. The physical condition of females after nesting was analysed by using the residual scores from the regression of log BMA to log SVL for the pooled data of 1995 and 1996 (see e.g. Madsen & Shine, 1999). Differences in CS between years were analysed by comparing the regression lines of CS on female SVL. An ANCOVA was used to analyse the variation in RCM in the two study years, in which ACM was the dependent variable, BMA was the covariate, and year was the class variable.

RESULTS

REPRODUCTIVE PHENOLOGY AND NEST CHARACTERISTICS

In 1995 we found 65 nests, and the nesting season extended from 25 September to 7 November, with a peak in mid October. In 1996 we found 53 nests and the nesting season extended from 19 September to 28 October, with a peak in the first week of October (Fig. 2). Mean egg-laying date differed significantly between years ($F_{1,116}=26.21$, $P<0.0001$), with females nesting earlier in the wet year.

Mean tunnel length was 52 cm (SD=16.7, range: 23–87, $n=14$) in 1995 and 39 cm (SD=15.17, range: 20–80, $n=53$) in 1996, and differed significantly between years ($F_{1,36}=6.51$, $P=0.015$). Nest depth averaged 32 cm (SD=7.4, range: 18–42) and 36 cm (SD=9.3, range: 15–60), respectively, but did not differ statistically between years ($P>0.05$).

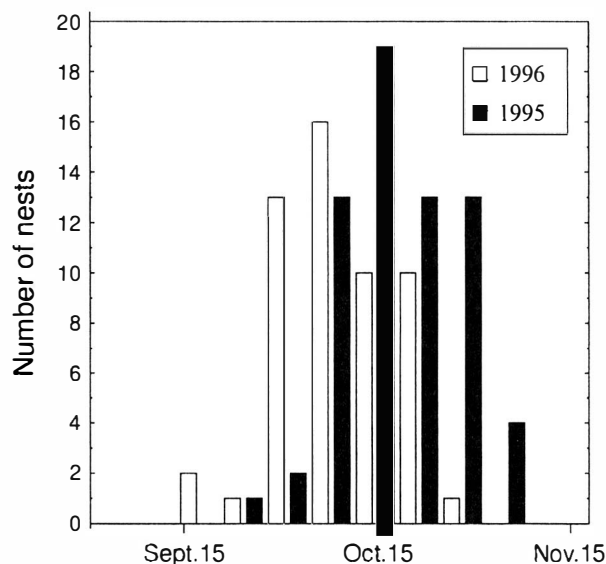


FIG. 2. Frequency distribution of the number of nests found each week during the period of study of reproduction by chameleons in southern Spain.

TABLE 1. Reproductive characteristics of female chameleons in 1995 and 1996, and the results of ANOVA comparing data from 1995 and 1996. (SVL: snout-vent length, BMB: body mass before oviposition, BMA: body mass after oviposition, ACM: approximated clutch mass = BMA-BMB, RCM: relative clutch mass= ACM/BMA, CS: clutch size).

	SVL (mm)	BMB (g)	BMA (g)	ACM (g)	RCM (%)	CS No. eggs
<i>1995</i>						
Mean	96.6	31.1	24.1	13.0	60.5	11.2
SD	11.2	8.6	7.2	6.5	29.8	4.4
Range	75-130	18-56	11.3-50.0	2-26	10.5-102.9	4-21
<i>n</i>	84	53	34	19	17	29
<i>1996</i>						
Mean	117.0	51.1	30.8	21.0	70.2	17.9
SD	11.1	13.3	59.8	7.1	17.7	6.4
Range	97-144	31.1-8.8	19.6-50.6	11.1-39.1	42.5-111.2	7-40
<i>n</i>	50	43	53	36	36	25
<i>F</i>	104.32	79.37	20.26	12.77	1.00	19.78
<i>P</i>	<0.005	<0.005	<0.005	<0.005	0.321	<0.0005
df	1,132	1,94	1,101	1,54	1,36	1,53

CHARACTERISTICS OF GRAVID FEMALES

Mean body size of reproductive females was smaller in the dry year than in the wet year (Table 1). The smallest gravid female in 1995 was 75 mm SVL (with BMA as low as 11.3 g) and 97 mm in 1996. The approximate clutch mass was smaller in the dry than in the wet year (Table 1), in accordance with the smaller body size of females. The clutch mass was correlated significantly with SVL (after logarithmic transformation of the two variables $r=0.731$, $P<0.0001$) and with BMA ($r=0.625$, $P<0.0001$) for data pooled over two years. For each

year, only the relationship between ACM and BMA in 1995 was not significant.

Female chameleons averaged a clutch mass relative to their body mass of 60.5% and 70.2% in 1995 and 1996, respectively. Some individuals in each year had a clutch mass as high as their own body mass ($RCM\geq 100\%$). Although females in 1995 were smaller and had a wider range of RCM than in 1996, we did not find a significant difference in mean RCM (Table 1). The ANCOVA with ACM as the dependent variable and BMA as covariate revealed a similar relationship between body mass and clutch mass in the two years (slopes: $F_{1,44}=0.539$, $P=0.467$; intercepts: $F_{1,44}=0.144$, $P=0.706$).

Clutch size ranged from 4 to 21 eggs in 1995 and from 7 to 40 eggs in 1996, and differed significantly between years (Table 1). Clutch size and SVL were correlated significantly in both years (1995: $r=0.659$, $P=0.0005$; 1996: $r=0.825$, $P<0.0005$). The slopes of the regression lines in 1995 and 1996 were not homogeneous (slopes: $F_{1,42}=17.800$, $P=0.0001$), indicating that CS increased more with SVL in the wet than in the dry year (Fig. 3).

The logarithmic regression of SVL and BMA was significant for pooled data from both study years ($R^2=0.509$), and residual scores did not reveal significant differences in body condition of females between 1995 and 1996. Within each year, the regression was also significant, with $R^2=0.263$ in 1995 and $R^2=0.645$ in 1996, and they differed significantly in their slopes (Fig. 4; slopes: $F_{1,91}=5.297$, $P=0.024$), suggesting that the lower BMA in the dry year was more related to the smaller body size of females than to environmental differences between years.

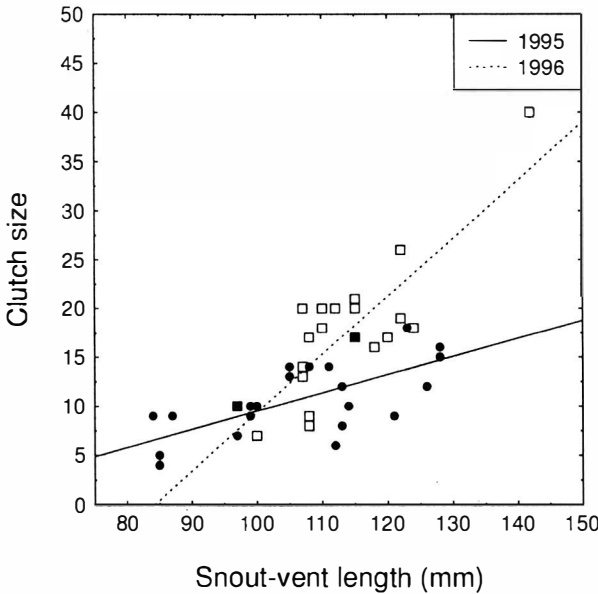


FIG. 3. Relationship between snout-vent length and clutch size of female chameleons in 1995 (filled circles) and 1996 (squares) in southern Spain.

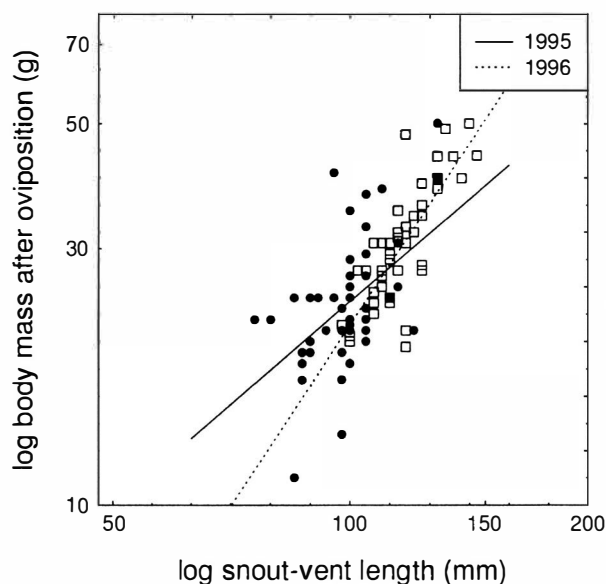


FIG. 4. Relationship between the logarithm of snout-vent length and body mass of female chameleons after oviposition, indicative of their physical condition (filled circles: 1995; squares: 1996).

MORTALITY OF NESTING FEMALES

Out of a total of 84 gravid females monitored in 1995, 14 were found dead: one was killed by a cat, two were run over by vehicles and 11 did not show external damage. We assumed the latter had died as a consequence of stress related to reproduction (see Blázquez *et al.*, 2000). In 1996, two out of 50 gravid females were found dead during the nesting season: one was run over, and the other was eaten by a snake while excavating the tunnel nest; no other dead females were found. The observed mortality was significantly different between years ($\chi^2=4.78$, $P=0.029$).

EGG CHARACTERISTICS

Egg variables did not differ between years but varied significantly among clutches (Table 2). Egg mass was positively related with EL ($r=0.610$, $P=0.0001$) and EW ($r=0.708$, $P<0.0001$), but not with egg shape. No correlation was observed between any of the egg variables with CS, SVL or BMA, but EW and egg shape were significantly related to RCM (EW: $r=0.479$, $P=0.02$; shape: $r=-0.445$, $P=0.033$).

DISCUSSION

Reproductive investment by female chameleons is relatively high. A single clutch is produced per year, which may reach 100% RCM in some individuals. Mean RCM obtained in this study ranks among the highest in reptiles, comparable to the RCM of viviparous terrestrial snakes (see Shine, 1992 for a review). Large clutch volumes and high RCM have mostly been described for reptiles with cryptic behaviour that use sit-and-wait foraging, for which the probability of escaping predators does not depend on rapid movements (Vitt & Congdon, 1978; Vitt & Price, 1982; Dunham *et al.*, 1988). *Chamaeleo chamaeleon* is a good example of a species for which a high RCM should not greatly influence either the probability of escaping predators or foraging efficiency, although the high volume of the clutch may decrease the ingestion rate of females during the late gravid period (Bons & Bons, 1960).

Environmental conditions apparently influenced the timing of reproduction; the nesting season of chameleons started later in the dry year than in the wet year. One explanation may be that females delayed oviposition in the dry year while waiting for better environmental conditions, and thus retained eggs in the oviduct for longer in 1995 than in 1996 (see Bons & Bons, 1960). The

TABLE 2. Descriptive statistics of chameleon egg length (EL, in mm), width (EW, in mm), mass (EM, in g), and shape (EL/EW) in 1995 and 1996, and results of ANOVA within and between years. Values were averaged per clutch, except for the range, which is given over the total egg number. n = number of clutches. *** $P<0.0005$; NS $P>0.05$.

	EL	EW	EM	Shape
1995				
Mean	16.82	10.49	1.16	1.60
SD	1.15	0.46	0.16	0.14
Range	13.7-21.3	8.6-11.7	0.8-1.5	1.4-2.0
n	19	19	19	19
$F_{18,199}$ (1995)	57.89***	22.52***	33.91***	
1996				
Mean	16.44	10.76	1.204	1.53
SD	0.91	0.53	0.20	0.09
Range	10.6-19.6	7.6-12.1	0.7-1.8	1.4-1.7
n	35	35	35	35
$F_{34,188}$ (1996)	7.59***	23.18***	43.23**	
$F_{1,48}$ (1995x1996)	1.2 ^{NS}	2.91 ^{NS}	0.73 ^{NS}	

1995 delay in the nesting season may also be related to the smaller size of females in that year. Cuadrado & Loman (1999) found that female size was correlated with reproductive timing, presumably because of a later attainment of sexual maturation in young (small) females, or because small females secure more resources for reproduction by extending the time that resources are allocated to follicles.

Nesting in chameleons often starts after the first autumn rains (Bons & Bons, 1960; Schleich, Kästle & Kabish, 1996), which probably favours nesting conditions and facilitates tunnel excavation in more compacted sandy soils. In our study, the nests were located deep in the substrate, which may insure adequate moisture conditions during the long incubation period. Difficulty in finding optimal nest conditions may be the reason for the excavation of longer tunnels in the dry year, although their depth was similar in the two years. Blasco *et al.* (1985) reported shorter tunnel length and lower nest depth for other localities from south-eastern Spain, which suggests that nest characteristics may vary among populations as a function of environmental conditions and soil characteristics.

Dunham (1983) demonstrated a correlation between the amount of rainfall and the availability of food resources to insectivorous lizards. Accordingly, we assumed that the drought conditions in 1995 reduced the availability of food resources in the study area. In 1995 reproductive females reached smaller body size than in 1996. Consequently, the interannual variation observed in CS and ACM is mainly explained by the variation in female body size, as fecundity is normally associated with this (see e.g. Roff, 1992).

In contrast, relative clutch mass did not differ significantly between years, and females made a similar investment in reproduction, independently of their body mass. In other species, the variation in RCM has been associated with different climatic conditions and resource availability (Vitt & Price, 1982). However, RCM remained remarkably constant for individuals of *Lacerta agilis* reared under both high and low resource availability (Olsson & Shine, 1997). A similar situation occurred in chameleons, for which the same investment is made by females in years of low and high availability of resources. However, the wide variation in RCM among chameleons suggests different responses by individual females, especially in the year of lower resource availability, which is supported by the lack of correlation in 1995 of ACM with BMA.

Egg characteristics (length, width and mass) remained constant despite the between-year variation observed in clutch size and female body size and mass. Egg variables were also similar to those described for other Spanish populations (Blasco *et al.*, 1985), which supports the idea of an optimal egg size (*sensu* Smith & Fretwell, 1974; Brockelman, 1975) for this species. According to optimal egg size theory, egg dimensions are limited by the minimum size that enables hatchling survival; the theory predicts that clutch size, rather than egg

and offspring size, varies with fluctuating resource availability (Brockelman, 1975). In our study the larger body size of female chameleons resulted in an increase in the number rather than size of eggs. Shape and width were the only egg variables related with RCM. Eggs were less elliptical in larger clutches, which could result from the effect of egg packaging inside the abdominal cavity.

The high mortality of reproducing females in 1995 can be explained either by a higher than optimal investment in reproduction by particular females, as suggested by the wide variation in RCM, or by their small body size which probably caused difficulties during egg-laying and even caused egg retention after oviposition (Blázquez *et al.*, 2000). These difficulties can be associated with the fact that a similar egg size is borne by small and big females.

Our results indicate that female reproductive investment by chameleons does not vary with environmental conditions, but under poor conditions females are smaller and consequently have lower clutch sizes than in years of high food resources. Chameleons produce a single clutch per year and their reproductive output is therefore constrained by the female abdominal cavity and physiological limitations. Under an overall perspective, the reproductive output of a chameleon population may be considered to vary according to environmental conditions, as lower fecundity and higher mortality of nesting females in dryer years than wet years results in lower juvenile recruitment.

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BODY MASS CONDITION IN GREEK TORTOISES: REGIONAL AND INTERSPECIFIC VARIATION

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Body mass and length data from large samples of wild *Testudo graeca*, *T. hermanni* and *T. marginata* in Greece were used to assess body mass condition. Mass-length relationships differed significantly between the sexes (females being heavier) and among the species (*T. marginata* being least heavy). Mass-length relationships for each species and sex were used to calculate the condition index (CI) $\log(M/M')$, where M is observed mass and M' is mass predicted from length, which is equal to residuals from the regression of $\log M$ on \log length. It was possible to use the empirical mass-length relationships from one population of *T. hermanni* to calculate CI in other populations of substantially different adult size. The seasonal pattern of the CI varied with latitude, with a sharper and later peak further north, and habitat, declining more in summer at a xeric coastal site. The seasonal patterns of CI in *T. graeca* and *T. marginata* were similar, with sharper and later peaks compared to *T. hermanni*. These seasonal patterns of CI were related to differences in activity and food availability among species and sites. The variability of the CI was similar in all three species, with most values between -0.1 and +0.1; seasonal variation was of relatively low amplitude, with a range of about 0.05 between the highest and lowest monthly means.

Key words: condition index, season, *Testudo graeca*, *Testudo hermanni*, *Testudo marginata*

INTRODUCTION

Many different parameters may be used to quantify the condition of an animal. Physiological variables are probably the most directly related to health but are often relatively difficult to measure, especially in chelonians which are capable of withdrawing within the margins of the shell when threatened (Jacobson, Behler & Jarchow, 1999). Blood, for example, may be examined for many variables related to health (Bonnet, 1979, Jacobson, 1987) and the results compared with normative values (Raphael *et al.*, 1994; Klemens *et al.*, 1997). Christopher *et al.* (1997) found that urea nitrogen content was a good measure of the hydration state of desert tortoises (*Gopherus agassizii*), and plasma iron, glucose and total protein were good indicators of their nutritional state. Blood may be sampled from the heart, jugular vein, brachial vein, ventral coccygeal vein, orbital sinus, or short-clipped toenails (Avery & Vitt, 1984; Jacobson, 1988, 1993; McDonald, 1976), but the procedure may be difficult and dangerous to a tortoise (Jacobson, Schumacher & Green, 1992), especially in the field. Other physiological parameters present even greater technical difficulties than collection and analysis of blood. For example, Henen (1991, 1997) measured the lipid content of live desert tortoises, but this required equilibration in a cyclopropane atmosphere for eight hours, analysis by gas chromatograph and calibration against total lipid extractions of dead tortoises.

A much simpler alternative is the mass of the tortoise in relation to its size (Jackson, 1980, 1991). Mass relative to size may be described simply as condition (for example, Blood & Henderson (1968) define normal bodily condition compared to obese, thin or emaciated animals), but this is better termed body mass condition to differentiate it from indexes based on other parameters. A further advantage of specifying body mass condition, rather than just condition or body condition, is that mass may not be linearly related to health. Very high body mass condition is likely to be an indication of poor health if an animal is obese or has egg peritonitis or fluid retention from renal or hepatic disease (Jackson, 1980; Lawrence, 1985; McArthur, 1996). As a related point, the word condition should be restricted to variables that reflect the health or physiological state of an individual, even if the relationship is not known in detail, rather than used as a short-hand description of morphological differences. The excellent study of Bonnet *et al.* (2001), for example, documented sexual dimorphism of carapace shape in *Testudo horsfieldii*, females being significantly wider and higher than males at the same length and having more bellied plastrons. There was also a significant sexual difference in mean mass adjusted for length, females being heavier. This was described as a difference in body condition and body condition index between the sexes, but only reflects the sexual shape dimorphism. Such differences in mass due to morphology have nothing to do with condition as such (Hailey, 2000: Fig. 3b) and should simply be described in terms of relative mass, not body condition.

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Body mass condition has been used with varying success in studies of chelonians. Jackson's results on *Testudo hermanni* and *T. graeca* have been widely used (Divers, 1996) and compared with other species of tortoise (Spratt, 1990). Nevertheless, the graphical study of McArthur (1996) showed no obvious differences between body mass of healthy and ill tortoises. Jacobson *et al.* (1993) did find a significant difference between healthy desert tortoises and those with respiratory disease, but identified six factors that limit the usefulness of body mass condition. These factors were related to potential differences in mass-length relationships (1) between the sexes; (2) in females before and after oviposition; (3) between the activity season and just after hibernation; (4) among populations due to differences in shape or dermal bone thickness; and the effects of (5) weight gain after drinking or (6) weight loss as faeces or urine on handling.

Nevertheless, equivalent difficulties occur with other parameters. Blood composition, for example, is known to vary with size, sex and season in chelonians (Hutton & Goodnight, 1957; Gilles-Baillien & Schoffeniels, 1965; Seidel, 1974; Frair & Shah, 1982; Taylor & Jacobson, 1982; Lawrence & Hawkey, 1986; Kim, Cho & Koh, 1987). Raphael & Jacobson (1997) also note that blood composition may vary among collection sites on the body due to differential contamination with lymph. Most of the difficulties with body mass condition can be overcome with the use of an appropriate mass-length relationship as reference. A previous study (Hailey, 2000) examined factors (1) and (3) of Jacobson *et al.* (1993), showing sexual and seasonal variation of body mass in *T. hermanni* at Alyki, a coastal site in northern Greece. There is also some information on factor (2) in this species; the presence of shelled eggs had no significant effect on body mass condition (Hailey & Loumbourdis, 1990), presumably because eggs reduce the space available in the abdomen and thus the volume and mass of gut contents (Meienberger, Wallis & Nagy, 1993). The carapace apparently limits relative clutch mass (mass of eggs/mass of body without eggs) in tortoises to about 5–10% (Hailey & Loumbourdis, 1988), in contrast to lizards where mean values greater than 50% are found in several species (Vitt & Congdon, 1978).

Testudo hermanni shows wide variation in adult body size in Greece (Willemsen & Hailey, 1999a) and it is unclear whether the empirical mass-length equations from Alyki are applicable to *T. hermanni* generally; for example, tortoises described by Meek (1985) were apparently much heavier at the same length. The first aim of this paper was to test the use of equations derived from tortoises at Alyki to calculate body mass condition in other populations of *T. hermanni*, and to examine the patterns of seasonal variation in different regions. The second aim was to derive mass-length equations for the other two species of tortoise in Greece, *T. graeca* and *T. marginata*, and then examine the seasonal variation of their body mass condition. These species differ in shape

from *T. hermanni*, especially *T. marginata* which is much narrower (Bringsøe, Buskirk & Willemsen, 2001), and are thus likely to require different reference equations. They also occupy contrasting habitats and are active with different body temperatures (Wright, Steer & Hailey, 1988; Willemsen, 1991), and are thus likely to be affected by season in different ways.

METHODS

Tortoises were found by walking through the habitat and were measured in the field (Stubbs *et al.*, 1984) and released immediately afterwards at the point of capture. Straight carapace length was measured to the nearest 1 mm; this is the horizontal straight distance between the front and rear of the carapace with the plastron flat on the substrate, as shown by Stubbs *et al.* (1984), McArthur (1996) and Bonnet *et al.* (2001). The mass of most tortoises was measured to the nearest 5 g with 2 kg or 3 kg Soehnle spring balances. Small individuals were measured to 1 g with a 250 g Soehnle spring balance. Sex was determined by plastral concavity and larger tails in males; only animals larger than 10 cm carapace length are considered here. *Testudo hermanni* may be sexed from 10 cm; the size at maturity varied substantially among sites (Willemsen & Hailey, 1999a) so the data are grouped into males and females here, rather than subadults, adult males and adult females as used previously (Hailey, 2000). Sex could not usually be estimated from external appearance in *T. graeca* <13 cm or *T. marginata* <17 cm. In these species one category was identifiable males, and all other tortoises larger than 10 cm formed the other category (females + subadults). The shape of subadult tortoises is generally similar to that of females (Stubbs *et al.*, 1984), and these could not be distinguished in *T. graeca* and *T. marginata* except by size. Each individual was permanently marked with a unique code by notching the marginal scutes with a file.

Populations of *T. hermanni* were grouped to obtain sufficient data for analysis of regional trends; maps of the locations and descriptions of the habitats of these sites have been given previously (Willemsen & Hailey, 1989, 1999a,b). Three regions were examined here: (1) the south, including low altitude sites in the Peloponnese (with mean mass of adult males at each site from Willemsen & Hailey, 1999a); Kalamata (0.47 kg), Sparta (0.51 kg) and Olympia (0.61 kg); (2) Meteora, a mid-latitude and mid-altitude site in central Greece with intermediate-sized tortoises (0.70 kg), where many individuals have been marked; (3) the north, including Deskati (1.36 kg), Kastoria (1.13 kg), Agios Dimitrios (0.90 kg), Mikri Volvi (0.89 kg) and Litochoron (0.76 kg). Data for *T. graeca* and *T. marginata* were from all sites where they were observed (Willemsen & Hailey, 1989: Table 2), excluding the single *T. graeca* at Olympia that was probably an introduction. Rainfall data were from meteorological stations at Sparta for the south, Kalabaka near Meteora, and Ptolemaïdos for the north (Willemsen & Hailey, 1999a: Fig. 2). Rainfall data for Alyki were from Trikala, near Eginion (this is a

different place from Trikkala, in central Greece, described previously by Willemssen & Hailey, 1999a).

Body mass condition was calculated from the body mass (M) of a tortoise compared to that predicted (M') from the relationship between mass and length (L) (after Le Cren, 1951). The mass-length relations which were used to calculate M' included each individual tortoise only once. These allometric equations were of the form $\log M' = \log a + b \log L$, which corresponds to $M' = aL^b$ in exponential form. The simpler condition factor K calculated using $b=3$ is unsuitable where shape or density changes with size, when the allometric equation is preferable (Le Cren, 1951). Mass-length relationships of different species or sexes were compared using analysis of covariance (ANCOVA) with $\log M$ as the dependent variable, species or sex as a fixed factor, and $\log L$ as covariate.

A previous study (Hailey, 2000) found that $\log (M/M')$ was the best the condition index (CI) based on body mass; this is equal to residuals from the regression of $\log M$ on $\log L$. $\log (M/M')$ is normally distributed, and allows analysis of interaction effects in analysis of variance (ANOVA). Values of $\log (M/M')$ were calculated with SPSS; the CI was also converted to a relative mass (M/M') for ease of interpretation, often expressed as a percentage. A tortoise with observed mass equal to predicted mass thus had $M/M' = 1.0$ or 100%, and $CI = 0$. Sexes or species were also compared using a relative mass expressed as a percentage; the ratio of their predicted (M'_a/M'_b) masses where a and b are the two groups. In this case the relative mass depends on morphological differences, not on condition as such, and should not be converted into a CI as noted in the introduction. Seasonal patterns of the CI were compared with two-way or three-way ANOVA with month and sex, region or species. These analyses used only one value of CI for each individual tortoise in each month (not replicated measures within the same month). The resulting F values are shown with main effects and residual degrees of freedom. Frequency histograms of CI values used these monthly data (Hailey, 2000).

RESULTS

TESTUDO HERMANNI

Observations of *T. hermanni* were made between April and October, although there was low activity in the north in September and October and no mass measurements were made then. Initial analyses used mass-length equations for male and female *T. hermanni* from Alyki in July (Hailey, 2000: Table 2) to calculate M' and the CI. The mean relative mass in each of the three regions was 102–103% (Fig. 1), only 1–2% different from values at Alyki calculated in the same way (i.e. using data from April to October with the July reference equations, giving a mean of 101%). The south and north had similar relative mass, and were only 1% different from that at Meteora. There was thus no evidence of major differences in mass-length relationships in *T. hermanni* among regions, despite the large difference of

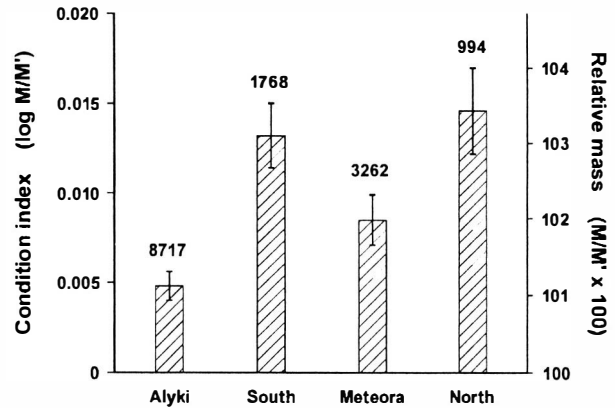


FIG. 1. Mean condition index of *T. hermanni* measured from April to October in different areas, using M' calculated from the mass-length equations for males and females from Alyki in July. The right y axis shows the CI converted to relative mass (%). Vertical lines show 95% confidence intervals, and numbers above bars show sample sizes.

mean adult size from south to north in Greece. The similarity of the CI values among sites is indeed remarkable given the independent observers and different equipment. A 1% difference in relative mass is similar to the level of precision of the field measurements; mass was recorded to the nearest 5–10 g for a 0.5–1 kg tortoise.

Three-way ANOVA of CI with month, sex and region used data from April to August only, because September and October data were not available for the north. There was a significant interaction of month \times sex ($F_{4,5511} = 6.93$, $P < 0.001$) showing different seasonal patterns of males and females, and of month \times region ($F_{8,5511} = 3.33$, $P = 0.001$) showing different seasonal pat-

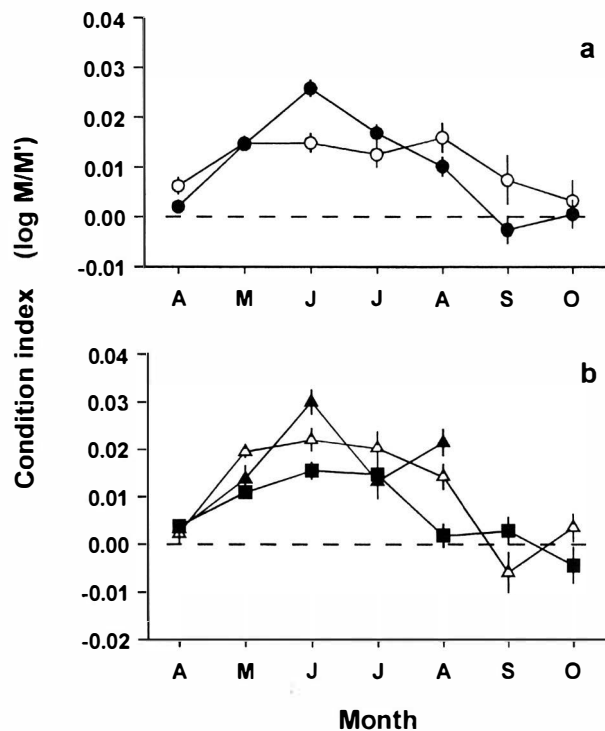


FIG. 2. The seasonal variation of condition index in *T. hermanni*. (a) Sexual differences; females (open circles), males (filled circles). (b) Regional differences; the south (open triangles), the north (filled triangles), Meteora (filled squares). Bars show \pm SE.

TABLE 1. Mass-length regression equations for *Testudo* species in Greece, and example predicted masses for comparison. Values are shown \pm SE; $\log a$ and b are the intercept and slope, respectively of the regression of $\log M$ (g) on $\log L$ (mm); n is the number of individuals; and r^2 is shown as a %. Separate equations are given for females (f) and males (m) >10 cm; for *T. graeca* and *T. marginata* the categories are females and subadults (f+s, which cannot be distinguished by morphology in these species), and males.

Species	$\log a$	b	n	r^2	Predicted mass at L		
					100	150	200
<i>T. hermanni</i> (f)	-3.188 \pm 0.030	2.774 \pm 0.014	1156	97.2	229	705	1567
<i>T. hermanni</i> (m)	-3.180 \pm 0.030	2.760 \pm 0.014	1948	95.3	219	670	1482
<i>T. graeca</i> (f+s)	-3.307 \pm 0.052	2.846 \pm 0.024	276	98.1	243	769	1745
<i>T. graeca</i> (m)	-2.549 \pm 0.110	2.498 \pm 0.049	239	91.5	-	771	1581
<i>T. marginata</i> (f+s)	-2.724 \pm 0.119	2.531 \pm 0.051	101	96.0	218	608	1259
<i>T. marginata</i> (m)	-2.476 \pm 0.160	2.420 \pm 0.068	116	91.7	-	617	1237

terns in the three regions. There was no significant interaction of month \times sex \times region ($F_{8,5511}=1.86$, $P=0.061$), showing that the difference between the sexes followed a similar seasonal pattern in all regions. The seasonal patterns of males and females are shown in Fig. 2a. The CI of males increased from April to reach a peak in June, then declined to a minimum in September. The CI of females showed less seasonal variation, with high values

from May to August. The relative condition of females ($CI_{\text{females}} - CI_{\text{males}}$) was lowest in June, and highest in September.

The seasonal patterns in the three regions (excluding Alyki) are shown in Fig. 2b. Although these patterns were significantly different, they showed the same general trends, with initially low CI in April, high values in spring and summer, and a decline in autumn (where data were available). There were no data for March when the CI was lowest at Alyki following emergence from hibernation (Hailey, 2000). There was no clear geographic trend of the seasonal pattern, apart from a tendency for the peak of the CI to become narrower and to occur later in the year from south to north. There was thus a plateau lasting from May to August in the south, a peak from May or June to July at Meteora, and a sharp peak in June, and possibly August, in the north.

TESTUDO GRAECA

There were fewer data available for *T. graeca* (or *T. marginata*) than for *T. hermanni*, and so the reference mass-length equations were derived using data from all months combined (but each individual only once). These equations are shown in Table 1, together with comparable equations for *T. hermanni* calculated in the same way (i.e. using the three regions, months combined but each individual included only once). ANCOVA of $\log M$ by sex with $\log L$ as covariate showed that the mass-length relationship differed significantly between the sexes in *T. graeca* ($F_{1,512}=9.72$, $P<0.001$) and in *T. hermanni* ($F_{1,3101}=232.9$, $P<0.001$). *Testudo graeca* were heavier than *T. hermanni* of the same length, in both females and subadults (Fig. 3a) and males. ANCOVA showed that the differences in mass-length relationships between these species were significant in both females and subadults ($F_{1,1429}=236.7$, $P<0.001$) and males ($F_{1,1392}=346.0$, $P<0.001$).

Seasonal variation of the CI in *T. graeca* is shown in Fig. 3b, compared to that for *T. hermanni* (the three regions combined). Two-way ANOVA of CI with month and species had a significant month \times species interaction ($F_{6,6691}=14.37$, $P<0.001$) showing that the seasonal pattern of CI differed between these species. The major difference was more pronounced seasonal

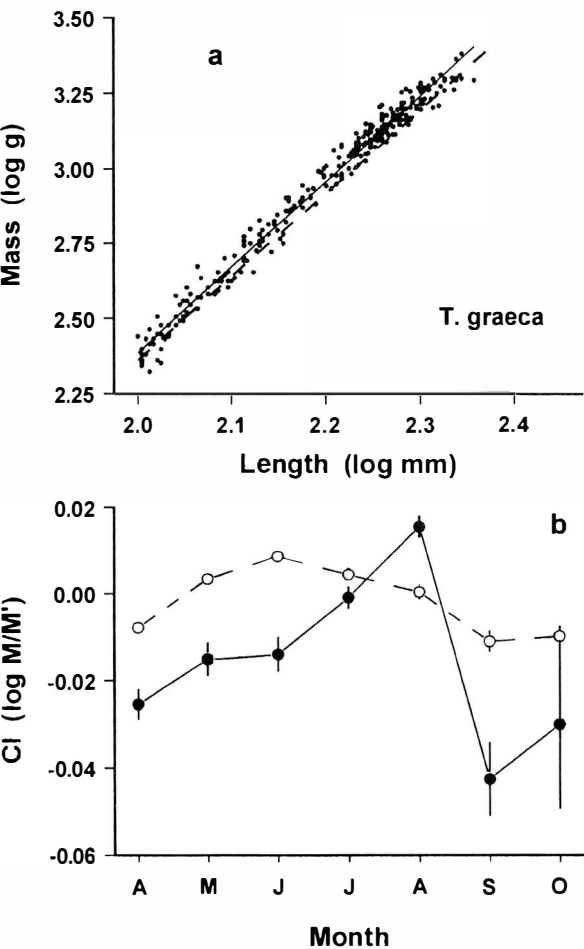


FIG. 3. (a) The relationship between mass and length in female and subadult *T. graeca*. The regression equation is given in Table 1; the dashed line shows the corresponding relationship for *T. hermanni*. (b) Seasonal variation of condition index in *T. graeca* (solid circles) and *T. hermanni* (open circles, all three regions pooled). Bars show \pm SE.

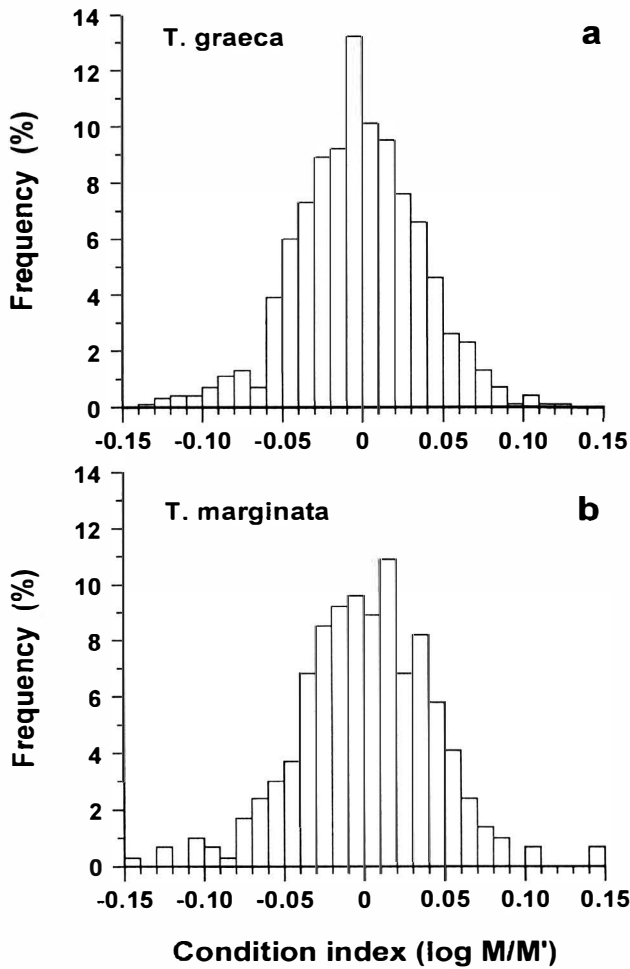


FIG. 4. Frequency distributions of condition index in (a) *T. graeca* and (b) *T. marginata* in the wild.

variation in *T. graeca*, the peak of the CI being sharper and occurring later in the year (in August, compared to June in *T. hermanni*). The standard deviation of CI in *T. graeca* was 0.039 ($n=695$), compared to 0.038 ($n=6024$) for *T. hermanni* (the three regions combined); the variability of CI was thus similar in the two species. The frequency distribution of CI values in *T. graeca* is shown in Fig 4a; most values were between -0.1 and +0.1.

TESTUDO MARGINATA

Sexual dimorphism of the mass-length relationship was only marginally significant in *T. marginata*, which is probably partly due to the small sample size for this species. The ANCOVA of $\log M$ with $\log L$ as covariate was not significant ($F_{1,214}=2.64$, $P=0.106$), but that of $\log L$ with $\log M$ as covariate was significant ($F_{1,214}=7.19$, $P=0.008$). The latter is perhaps better when comparing animals of different shape, which will be equivalent at the same body mass rather than the same length. Analyses of mass on length are generally used here, however, because the CI requires prediction of mass and this should thus be the dependent variable. *Testudo marginata* had lower mass than *T. hermanni* of the same length, in both females and subadults (Fig. 5a)

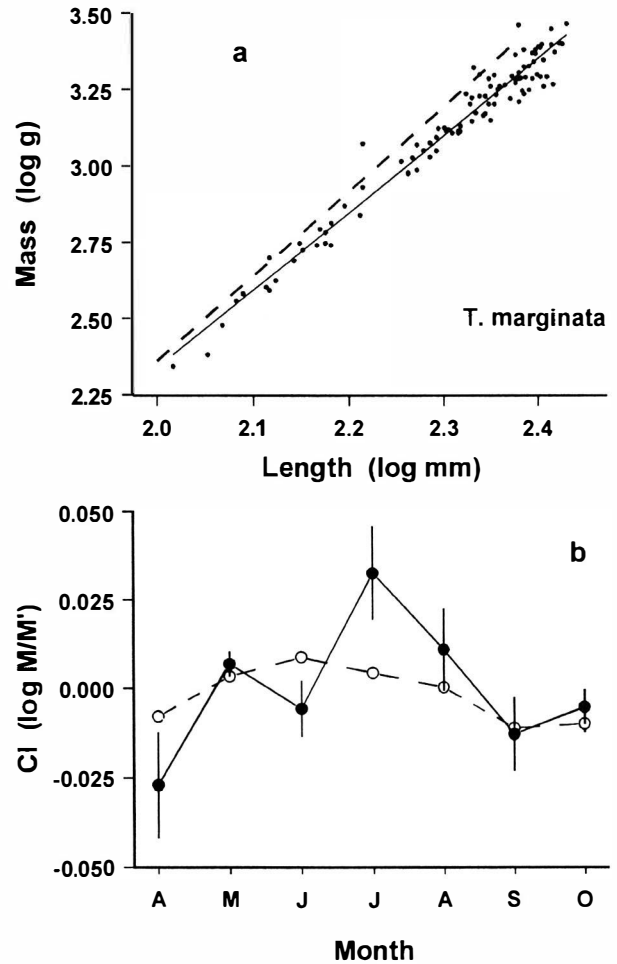


FIG. 5. (a) The relationship between mass and length in female and subadult *T. marginata*. The regression equation is given in Table 1; the dashed line shows the corresponding relationship for *T. hermanni*. (b) Seasonal variation of condition index in *T. marginata* (solid circles) and *T. hermanni* (open circles). Bars show \pm SE.

and males. ANCOVA showed that the differences in mass-length relationships between these species were significant in both females and subadults ($F_{1,1254}=445.9$, $P<0.001$) and males ($F_{1,2061}=454.1$, $P<0.001$).

The mass-length relationships (Table 1) are as expected from the morphology of the three species. Quantitative comparisons are shown most clearly by predicted mass at three representative lengths. Subadults of the three species differed rather little at 100 mm, with a range of predicted mass of only 10% from about 220-240 g. Juvenile and subadult *Testudo* of all species generally have a similar shape (Bringsøe *et al.*, 2001) which only diverges with growth. Female *T. hermanni* were about 5% heavier than males of the same length, as reported previously (Hailey, 2000). The other two species may only be sexed from external morphology at larger sizes. Male *T. graeca* had similar mass to females at 150 mm but became progressively lighter than females, with a lower value of b . The narrow *T. marginata* diverges most from the standard tortoise shape (Bringsøe *et al.*, 2001), with the lowest values of b , but also showed low sexual dimorphism. In contrast

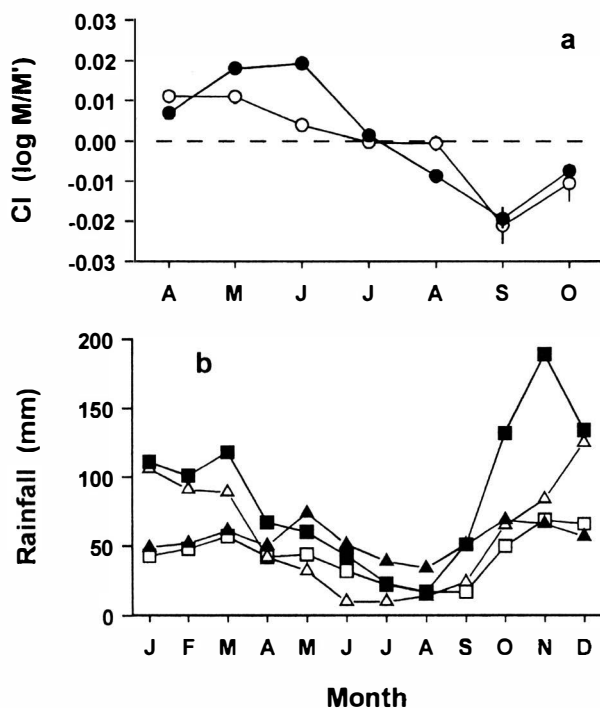


FIG. 6. (a) Seasonal variation of condition index of *T. hermanni* at Alyki; females (open circles), males (filled circles), bars show \pm SE. (b) Seasonal variation of rainfall in different areas; the south (open triangles), the north (filled triangles), Meteora (filled squares), Alyki (open squares).

to their similarity at 100 mm, predicted mass of the three species differed substantially at 200 mm (Table 1). The relative mass using M' showed *T. graeca* to be 9% heavier than *T. hermanni* (average of females and males), and *T. hermanni* 22% heavier than *T. marginata*, at 200 mm.

The seasonal pattern of CI in *T. marginata* is compared with that of *T. hermanni* in Fig. 5b. Two-way ANOVA of CI with month and species showed a significant month \times species interaction ($F_{6,6303}=2.48$, $P=0.021$), with *T. marginata* having more pronounced seasonal variation and a sharper and later (in July) peak of CI. The seasonal pattern of CI was thus similar in *T. marginata* and *T. graeca*, and two-way ANOVA of CI with month and species showed no significant month \times species interaction between these two species

($F_{6,960}=1.79$, $P=0.099$). The standard deviation of CI in *T. marginata* was 0.046 ($n=293$); the CI was thus slightly more variable than in *T. graeca* or *T. hermanni* but most values were still between -0.1 and +0.1 (Fig. 4b).

DISCUSSION

REGIONAL VARIATION

The first question in *T. hermanni* was whether a single mass-length relationship could be used for populations from different regions with substantially different adult body size. Differences in relative mass among regions were only in the order of 1-2%. Mass-length relationships are thus very consistent provided that length is measured in a standard way. Other straight length measurements are possible, such as along the midline (shorter than the straight carapace length) or inclined downwards (longer than a horizontal measurement). These differ by up to about 5% in an individual *T. hermanni*, but would lead to greater differences in the CI; a 5% difference in L would lead to a 12-15% difference in M' (with the range of b in Table 1) and thus change the CI by 0.05-0.06. Differences in measuring L can thus affect the CI as much as disease (Willemsen *et al.*, 2002) or seasonal variation.

Body mass condition could reflect the level of hydration of a tortoise, the fullness of its gut, or the composition of body tissues, particularly changes in the mass of fat and the shell. The relative density (compared to water = 1.0) is about 0.90 for body fat, 1.10 for fat-free body tissues, and 3.0 for bone mineral (Blaxter, 1989). The volume of a tortoise is relatively constant, determined by the shell, with little room for expansion around the limb openings. Increased fat content may therefore be at the expense of other tissues of higher relative density, which would reduce the CI, unlike most animals in which increased fat content is associated with higher body mass. The Quetelet index in man for example, of mass : height², is directly related to fat content (Blaxter, 1989). Increased bone mineral content would increase the CI. The fat content of chelonians is, however, rather constant (Brisbin, 1972) and there is little information on seasonal mineral cycles. Most seasonal variation of the CI observed here is therefore attributed

TABLE 2. Seasonal mass-length regression equations for *T. hermanni* >10 cm at inland sites in Greece. Values are shown \pm SE; log a and b are the intercept and slope, respectively of the regression of log M (g) on log L (mm), n is the number of individuals, and r^2 is shown as a %.

	Females				Males			
	log a	b	n	r^2	log a	b	n	r^2
April	-3.106 \pm 0.050	2.734 \pm 0.023	565	96.3	-3.056 \pm 0.042	2.699 \pm 0.019	1180	94.2
May	-3.132 \pm 0.044	2.750 \pm 0.020	730	96.2	-3.042 \pm 0.044	2.698 \pm 0.021	1072	94.1
June	-3.293 \pm 0.053	2.822 \pm 0.024	395	97.3	-3.330 \pm 0.065	2.836 \pm 0.030	441	95.4
July	-3.154 \pm 0.075	2.758 \pm 0.034	206	97.0	-2.881 \pm 0.085	2.626 \pm 0.039	446	91.1
August	-3.253 \pm 0.086	2.805 \pm 0.039	121	97.8	-3.219 \pm 0.084	2.778 \pm 0.039	385	93.1
September	-3.148 \pm 0.155	2.753 \pm 0.071	55	96.5	-3.288 \pm 0.140	2.804 \pm 0.064	167	91.9
October	-3.117 \pm 0.139	2.737 \pm 0.063	67	96.6	-3.110 \pm 0.134	2.723 \pm 0.062	194	90.9

to the level of body hydration and the fullness of the gut - and these two factors will be correlated where most water comes from the food. Gilles-Baillien & Schoffeniels (1965) found that the osmotic pressure of the blood of *T. hermanni* reached a minimum in June and July, and was maximal at the end of hibernation. This is opposite to the pattern of body mass condition, which suggests that at least part of the variation of the CI was due to changes in hydration state (high body water content causing low osmotic pressure and *vice versa*).

There were significant differences in the seasonal pattern of CI among the three regions that could be due to two sets of variables; habitat and food availability, or activity and thermoregulation. Rainfall is highest in winter in all parts of Greece (Fig. 6), so spring vegetation is lush and food availability is high in all regions and habitats. The delayed peak of CI further north is thus likely to be due to differences in activity/thermoregulation rather than habitat/food availability. In particular, thermoregulation by basking in spring becomes increasingly important in *T. hermanni* from south to north in Greece (Willemsen & Hailey, 1999b). Time spent basking may limit early feeding activity in the north and delay the timing of maximum CI there.

One reason for the slightly lower annual mean CI at Alyki (Fig. 1) is the different seasonal cycle, shown in Fig. 6a in the same form as for the three regions described here. The CI at Alyki was similar to that in the other three areas from April to June, ranging from 0 to 0.02. The CI decreased after June at Alyki, but remained high until July or August in other areas. The different pattern at Alyki is unlikely to be due to activity/thermoregulation (which was similar to the mid-latitude site at Meteora) or climate (since all areas had lowest rainfall from June to September; Fig. 6b). The difference is more likely to be due to habitat/food availability. Alyki was a relatively xeric habitat with sandy soil and no surface water after spring, so food availability declined in summer in most parts of the site. Although the same mass-length equations may be used to calculate a CI for *T. hermanni* from different regions, those from Alyki are not the best for detailed studies because of this atypical seasonal pattern. Monthly regression equations for the pooled data for the south, Meteora and north are provided in Table 2 to allow calculation of a seasonally adjusted condition index (CI_s) for comparisons among sites or years sampled in different months. These equations have been found to be more suitable for measuring the body mass condition of *T. hermanni* at other sites (Willemsen & Hailey, 2001).

Figure 6a also shows that the seasonal pattern of the difference between the sexes at Alyki was similar to that in other areas; the CI of females relative to males was lowest in June during the nesting season. The relatively low CI of females in June might simply be due to recent oviposition, but could also reflect different activity of the sexes. Females move further (Hailey, 1989; Longepierre, Hailey & Grenot, 2001) and are sighted more frequently (Hailey & Willemsen, 2000) than males

in June, and this activity may occur at the expense of feeding. Food consumption of females is in any case likely to be low in the nesting season as eggs reduce the volume available for gut contents (Meienberger *et al.*, 1993), although this will not affect the CI unless the densities of eggs and gut contents differ substantially.

INTERSPECIFIC COMPARISONS

The mass-length relationships of the species were as expected from their shapes (Bringsøe *et al.*, 2001), with *T. marginata* being the lightest at a given length. The equations in Table 1 may be used to calculate the CI of captive European tortoises (Willemsen *et al.*, 2002). That paper describes the slight difference (of 3% relative mass) in mass-length relationships between the two subspecies of *T. hermanni*. Lambert (1982: Fig. 2) showed that there were no obvious differences between mass-length relationships of *T. graeca* from North Africa (*T. g. graeca*) and the Eastern Mediterranean (*T. g. iberica*). There are no subspecies of *T. marginata*, although the dwarf species described by Bour (1996) in the Peloponnese could be regarded as such (Bringsøe *et al.*, 2001).

Meek (1985: Fig. 6) also examined the mass-length relationships of these species (sexes combined) and reported that *T. hermanni* from Yugoslavia were substantially heavier than *T. graeca* or *T. marginata*. The equations for *T. graeca* (from Lambert, 1982) and *T. marginata* (from a reanalysis of data from Hine, 1982) give similar body masses to those found here; for example, 1501 g and 1257 g, respectively, at 200 mm, whereas the equation for *T. hermanni* gives a substantially higher body mass of 2390 g at 200 mm. The latter is 57% higher than the mass of 1525 g predicted from the average of males and females from Greece in this study (Table 1). In contrast, populations of different body size in Greece, and wild tortoises from Italy and France (Willemsen *et al.*, 2002), differed in relative mass by only up to 3%, and the seasonal peak of CI was only about 0.03 in any region or species. The discrepancy between the length-specific mass of *T. hermanni* in Yugoslavia and elsewhere cannot now be resolved (R. Meek, personal communication), but it is potentially important.

The annual cycle of CI in *T. graeca* and *T. marginata* differed from that in *T. hermanni*, with peaks occurring in summer rather than in spring. This pattern corresponds well with the activity of *T. graeca* at Alyki, the only site in Greece where this species has been studied throughout the year. *Testudo graeca* emerged later from hibernation, in April compared to March in *T. hermanni*, and occupied open coastal vegetation that had relatively little food in early spring. Food availability was greater in coastal vegetation than other habitats in summer, when some *T. hermanni* moved into coastal areas (Wright *et al.*, 1988). *Testudo graeca* also had higher body temperatures (Wright *et al.*, 1988) and larger body size (Hailey & Loumbourdis, 1988) and

thus greater thermal inertia than *T. hermanni*. Both of these factors would increase the need to bask in spring (Lambert, 1981) and limit the time available for other activities. The low CI of *T. graeca* in spring in Greece may be explained by both activity and food availability being low, but increasing in summer to give a late peak of CI. This species usually does not aestivate in Greece (although summer activity may be low in some years), unlike arid regions of Iran (Pritchard, 1966) or southern Spain (Diaz-Paniagua, Keller & Andreu, 1995).

The seasonal pattern of CI in *T. marginata* is less easy to understand. In many respects, *T. marginata* is similar to *T. graeca*, using more xeric habitats than *T. hermanni* and having higher body temperatures in the wild (Willemsen, 1991). Studies of wild tortoises suggest that thermoregulation of *T. marginata* is similar to that of *T. graeca*, so a summer peak of CI might be expected, owing to constraints of cool conditions in spring. Studies of *T. marginata* in captivity, however, show that this species is able to be active with low body temperatures in spring, in contrast to *T. graeca*. Panagiota & Valakos (1992) found that *T. marginata* in an outdoor enclosure in southern Greece did not seek refuges in winter but were inactive above ground, becoming active on warm days, while *T. hermanni* hibernated buried in soil. They found no significant differences between the body temperatures of the two species, but the range was greater in *T. marginata* (8.0–34.7 °C) than *T. hermanni* (14.7–33.3 °C). Captive *T. marginata* in Italy were also often active in early spring with low body temperatures (R. E. Willemsen, personal observations). The low CI of *T. marginata* in spring is therefore surprising in view of this capacity for low-temperature activity. Further study of the ecology of this interesting species in the wild is clearly required.

The presence of significant seasonal variation of the CI shows that body mass condition is of interest in ecological studies, especially as it is simple to measure with minimal disturbance to the tortoise. The CI may also be valuable for management of captive tortoises (Willemsen *et al.*, 2002). The six factors considered by Jacobson *et al.* (1993) to limit the usefulness of body mass condition may now be evaluated for the species studied here. Sexual differences in mass-length relationships (1) have been confirmed in all three species of *Testudo*, but only cause a problem if the CI is calculated from regressions pooling the sexes (Hailey, 2000: Fig. 3b). The use of separate regressions for males and females to calculate M' , and thus the CI, compensates for these differences. Changes in body mass before and after oviposition (2) do not cause a difference between the CI of females with and without shelled eggs (Hailey & Loumbourdis, 1990) and thus seem to be minimal in *Testudo*, offset by changes in gut contents (Meienberger *et al.*, 1993). Seasonal changes in the CI (3) have been demonstrated, but are of small amplitude compared to the overall variation of the CI; seasonal variation had a maximum range of about 0.05 between the highest and lowest months. Seasonal variation may be removed

from the CI by using mass-length relationships for different months (Table 2) where sufficient data are available, or by simply comparing with known seasonal patterns.

Changes of mass after drinking (4) or voiding large amounts of faeces or urine (5) remain a problem. The possible effect of drinking is best evaluated by considering the availability of standing water sources or recent rainfall; this would in any case affect condition estimated from blood composition. The effects of voiding faeces or urine are best minimised by handling tortoises quickly and carefully and in a standard way; mass should be measured, for example, before any more disturbing handling such as marking the shell. Methods to obtain urine-free mass are available, but the precautions and delay involved largely negate the advantage of body mass condition as a practical measure. For example, treatment with pilocarpine (Dorando, 1979) successfully eliminated stored fluids but required 20 min to be effective, plus the need for sterile technique and the possibility of disturbance to subsequent behaviour. Short-term variation in body mass would be averaged out in a large sample; the best way to minimise this in an individual tortoise would be to use the mean mass measured over a few days.

Geographic variation in mass-length relationships (6) caused by differences in shell shape or dermal bone thickness did not affect the CI of *T. hermanni* in Greece, even though mean adult size varied substantially among populations. There was also little variation between subspecies of *T. graeca* (Lambert, 1982) or *T. hermanni* or between wild and healthy captive tortoises from different areas (Willemsen *et al.*, 2002). The overall mass-length relationships of male and female *T. graeca*, *T. hermanni* and *T. marginata* given in Table 1 are thus a good basis for measuring body mass condition. A facility for calculating CI for tortoises of these species of carapace length 100 mm or larger is provided at <http://www.ahailey.f9.co.uk/cond.htm>. Further accuracy may be achieved by using monthly mass-length relationships or by considering the seasonal pattern of CI. In conclusion, body mass condition is a useful variable for large samples of tortoises, from field studies or large captive collections. Results for individual tortoises should be used in conjunction with other indicators of health (Jacobson *et al.*, 1993).

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BODY MASS CONDITION AND MANAGEMENT OF CAPTIVE EUROPEAN TORTOISES

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The condition index (CI) of the tortoises *Testudo graeca*, *T. hermanni hermanni*, *T. h. boettgeri* and *T. marginata* was examined in captivity in southern and northern Europe. The CI was calculated using mass-length relationships of wild tortoises: $\log(M/M')$, where M is observed mass and M' is mass predicted from length. The mass-length relationships differed slightly between the subspecies of *T. hermanni*. Captive tortoises at the Centro Carapax (Italy) and the Oosterbeek Tortoise Study Centre (The Netherlands) had CI within the same range as wild tortoises, so there was no general effect of captivity on body mass condition even at densities ten times the highest observed in the wild. However, the seasonal pattern of CI at Oosterbeek differed significantly from that of wild tortoises, with a peak in late summer rather than in spring. Low CI of tortoises in some enclosures at the Centro Carapax prompted supplementary feeding before their health was affected. Tortoises at SOPTOM (France) during a period of disease had significantly lower CI than wild tortoises, with mean CI of -0.04 and mean relative mass (M/M') of 91%. The CI offers a useful guide to the health and management of captive Mediterranean tortoises, although further data are required on those kept in different circumstances, such as outdoor-only enclosures in northern Europe.

Key words: captivity, condition index, management, *Testudo*, tortoise

INTRODUCTION

There are many possible measures of condition in animals, the simplest of which is the body mass condition that compares mass with size (reviewed in Hailey, 2000; Willemsen & Hailey, 2002). A useful condition index (CI) for tortoises is $\log(M/M')$, where M is observed mass, M' is mass predicted from length (L), and M/M' is relative mass (usually expressed as a percentage). $\log(M/M')$ is equal to a residual from a regression of $\log M$ on $\log L$. A value of $CI=0$ indicates that observed mass = predicted mass (i.e. $M/M'=1.0$ or 100%), whereas a negative value of the CI indicates that observed mass is lower than predicted mass. Previous studies have shown that the CI in wild tortoises varies among species and areas with characteristic seasonal patterns (Willemsen & Hailey, 2002). Veterinary studies have also shown that body mass varies with health (Jackson, 1978, 1980, 1985): a relative mass less than about 80% is an indication of poor health (Hailey, 2000). The veterinary studies cited above used data from captive tortoises to calculate M' . It is not clear whether mass-length relationships of wild tortoises are applicable to captive animals, or whether the latter generally suffer from chronic underweight (e.g. from stress

or inadequate food) or obesity (e.g. from inactivity or excessively rich diet).

This paper deals with body mass condition in three large collections of captive tortoises, two in southern Europe and one in northern Europe. The two southern collections were in enclosed natural vegetation near to wild populations, permitting a good test of the effects of captivity (including all the conditions associated with captivity such as social stresses or disease transmission from crowding, diet, and food supply) separate from those of climate and other aspects of habitat. The seasonal variation of the CI in northern Europe, where the climate is less suitable for tortoise activity (Lambert 1981, 1983), was also of interest. We also examine the mass-length relationship in *Testudo hermanni hermanni* (formerly *T. h. robertmertensi*; Bour, 1986). This western subspecies is vulnerable (IUCN, 2000) and susceptible to habitat fragmentation (Longepierre, Hailey & Grenot, 2001) and loss of habitat (Guyot & Clobert, 1997), while the eastern subspecies, *T. h. boettgeri*, is more widespread and abundant (Willemsen & Hailey, 1989) and not currently listed as threatened (IUCN, 2000). Measurement of condition in *T. h. hermanni* is particularly important in view of controversial collection and release programmes (Devaux, 1990) in which large numbers of wild tortoises pass through captivity before relocation. *T. h. hermanni* is thought to differ slightly in shape from *T. h. boettgeri* (Ernst & Barbour, 1989); if the mass-length relationships differ

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TABLE 1. Details of enclosures, stocking densities and subspecies used to compare body mass condition for captive European tortoises in different study centres: Carapax (Italy); Oosterbeek (The Netherlands); SOPTOM (France).

Centre	Species	No. enclosures	Area (ha)	Density (ha ⁻¹)	Date of measurements
Carapax	<i>T. h. hermanni</i>	2	0.7	170-210	April-May 2000
Carapax	<i>T. h. boettgeri</i>	1	0.025	600	April-May 2000
Carapax	<i>T. marginata</i>	1	0.7	60	April-May 2000
Carapax	<i>T. graeca</i>	2	0.015-0.15	400-1000	April-May 2000
Oosterbeek	<i>T. h. boettgeri</i>	1	0.03	1100	April-Oct. 1980, 1985
SOPTOM	<i>T. h. hermanni</i>	3	0.03	2000-3300	July-Oct. 1996

significantly, then separate reference equations would be required to calculate M' and the CI in the two subspecies.

METHODS

Wild tortoises were measured in the field as described by Stubbs *et al.* (1984) and released immediately afterwards at the point of capture. Straight carapace length was measured to the nearest 1 mm on a flat-bed scale. The body mass of most tortoises was measured to the nearest 5 g with 2 kg or 3 kg Soehnle spring balances. Small individuals were measured to 1 g with a 250 g Soehnle spring balance. Tortoises in France were measured to 0.01 g on an electronic balance. Sex was determined by plastral concavity and relative tail size; only tortoises larger than 10 cm straight carapace length are considered here. Data were analysed with SPSS and Minitab for regression, analysis of variance (ANOVA) and covariance (ANCOVA), and comparison of a sample mean against an expected value (i.e. CI=0, when mean observed mass = predicted mass) using single-sample *t*-tests. In ANCOVA we used log M as the dependent variable, log L as the covariate, and sex or subspecies as a fixed factor. The variability of CI values was compared using Bartlett's test of homogeneity of variance (Sokal & Rohlf, 1981). Data on captive tortoises were from three sites and the stocking densities at each site are shown in Table 1.

THE CENTRO CARAPAX, ITALY

Tortoises were housed in enclosures containing their normal habitat of grassland, scrub and open woodland, on a hill slope. CI measurements of *T. h. hermanni* were made in two enclosures with stocking densities of about 200 ha⁻¹ (the highest population density found in wild *Testudo* is about 100 ha⁻¹; Stubbs *et al.*, 1985). One enclosure was based on an open field with encroachment of *Rubus*, coarse grasses, and macchia of the oaks *Quercus cerris* and *Q. ilex*, while the other was a former olive grove with *Rubus* scrub. The *T. h. boettgeri* measured here were kept in a grassland enclosure. *T. marginata* were in an enclosure of which a third was oak wood and the rest was olive trees and *Rubus* scrub; this enclosure had been occupied for 10 years and food plants were scarce and even the grass (not usually eaten by *Testudo*) was cropped short. *T. graeca* were kept in

different enclosures according to their origin, in vegetation similar to that for *T. h. hermanni*. Natural food plants such as *Medicago*, *Trifolium*, *Rumex*, *Plantago* and various Compositae were reduced in the enclosures due to tortoise grazing. All food plants had been eliminated from the *T. h. boettgeri* enclosure, which contained only grass; these tortoises were fed with fruits and sometimes vegetables (mostly *Lactuca sativa* and *Cichorium andivia*). Captive individuals of *T. h. hermanni*, *T. h. boettgeri*, *T. graeca* and *T. marginata* were measured and weighed in the last week of April and the first week of May 2000. Captive *T. h. hermanni* were compared with 159 female and 109 male *T. h. hermanni* from a local, wild population, measured in the same season between 1990 and 1995; several years' data were used to reduce the effect of variation of the CI among years. Each tortoise was considered only once. Further details of Carapax can be found at the web site <http://www.novars.it/carapax/>.

THE TORTOISE STUDY CENTRE, THE NETHERLANDS

Tortoises were kept out of doors in a large garden at Oosterbeek, with plexiglass domes and a small glass-house to which the animals had free access for thermoregulation. Their food was growing plants, supplemented with vegetables, fruit and bread. Almost all food plants were natural, or closely related to those eaten in the wild, such as *Trifolium*, *Sedum*, *Taraxacum* and *Plantago* species. Tortoises hibernated out of doors, either in natural refuges or in boxes filled with dead leaves or straw. Captive *T. h. boettgeri* were measured and weighed monthly between April and October in 1980 and 1985. A total of 26 tortoises (15 females and 11 males) were measured at different times; each tortoise is considered only once in each month. Further details of the Tortoise Study Centre can be found at the web site <http://www.phys.uu.nl/~eendebak/schild/schild.html>.

THE STATION D'OBSERVATION ET DE PROTECTION DES TORTUES DES MAURES (SOPTOM), FRANCE

Tortoises were kept in outdoor enclosures with low scrub of *Cistus* and *Erica*, but few food plants, and were fed vegetables and fruits. Captive *T. h. hermanni* were measured from July to October 1996. Comparative data

TABLE 2. Condition index (CI) of captive European tortoises. *n* is the number of individuals, except at Oosterbeek where this is the number of individual x month observations. *t* is the result of a single sample *t* test with the probability (*P*) that mean CI=0. Females (f) and males (m) are shown separately where there was a significant difference in CI between the sexes.

Group	Location	mean CI	<i>n</i>	SD	<i>t</i>	<i>P</i>
<i>T. h. hermanni</i>	Carapax	-0.0005	99	0.0443	0.12	0.90
<i>T. h. boettgeri</i>	Carapax	0.0177	14	0.0800	0.68	0.51
<i>T. graeca</i> (f)	Carapax	-0.0547	15	0.0407	5.04	<0.001
<i>T. graeca</i> (m)	Carapax	0.0018	9	0.0535	0.09	0.93
<i>T. marginata</i>	Carapax	-0.0229	20	0.0476	2.10	0.047
<i>T. h. boettgeri</i>	Oosterbeek	0.0057	175	0.0391	1.91	0.057
<i>T. h. hermanni</i>	SOPTOM	-0.0421	43	0.0462	5.98	<0.001

from wild tortoises were from Vidauban and Cannel des Maures, Var, France (described by Longepierre & Grenot, 1997) measured from July to October 1997-1999. Each individual was considered only once. Further details of SOPTOM can be found at the web site http://www.tortues.com/index_uk.html.

RESULTS

SUBSPECIFIC DIFFERENCES IN *T. HERMANNI*

The mass-length relationships of wild *T. h. hermanni* in Italy and *T. h. boettgeri* in Greece were compared by ANCOVA of log *M* by subspecies with log *L* as covariate. There was a significant difference between the two subspecies for females ($F_{1,1309}=10.14$, $P=0.001$) but not for males ($F_{1,2054}=1.66$, $P=0.197$), with females being about 3% heavier in Italy than in Greece at the same length. The CI of *T. h. hermanni* was calculated using the mass-length relationships for wild tortoises from Italy. An ANCOVA of log *M* by sex with log *L* as covariate showed a significant difference between the sexes ($F_{1,262}=14.99$, $P<0.001$). Separate reference equations were therefore used for females and males as follows:

Females, $\text{Log } M = -3.161 + 2.767 \log L$
(SE intercept=0.108, $\text{SE}_b=0.050$, $n=156$, $r^2=95.2\%$)

Males, $\text{Log } M = -3.610 + 2.967 \log L$
(SE intercept=0.238, $\text{SE}_b=0.114$, $n=109$, $r^2=86.3\%$).

The overall standard deviation of the CI was 0.038 ($n=265$). The variability of the CI in wild *T. h. hermanni* was thus identical to that of *T. h. boettgeri* in Greece, which also had $\text{SD}=0.038$ (Willemsen & Hailey, 2002).

CENTRO CARAPAX

The CI of captive *T. h. hermanni* at the Centro Carapax was calculated using the mass-length equations for wild tortoises from Italy (above). The CI did not differ significantly between females and males ($F_{1,97}=0.009$, $P=0.997$). The mean CI for captive tortoises was not significantly different from 0 (Table 2) (this mean is not necessarily equal to 0 because the CI was calculated using the regression for wild, not captive, tortoises). The mean CI for captive tortoises was also

not significantly different from the mean CI of wild *T. h. hermanni* in Italy ($F_{1,362}=0.024$, $P=0.97$). Most CI values of captive *T. h. hermanni* at the Centro Carapax were between -0.1 and +0.1 (Fig. 1a), and the variability of CI was similar to that of wild tortoises in Italy (Bartlett's test, $\chi^2=3.82$, $\text{df}=1$, $P>0.05$).

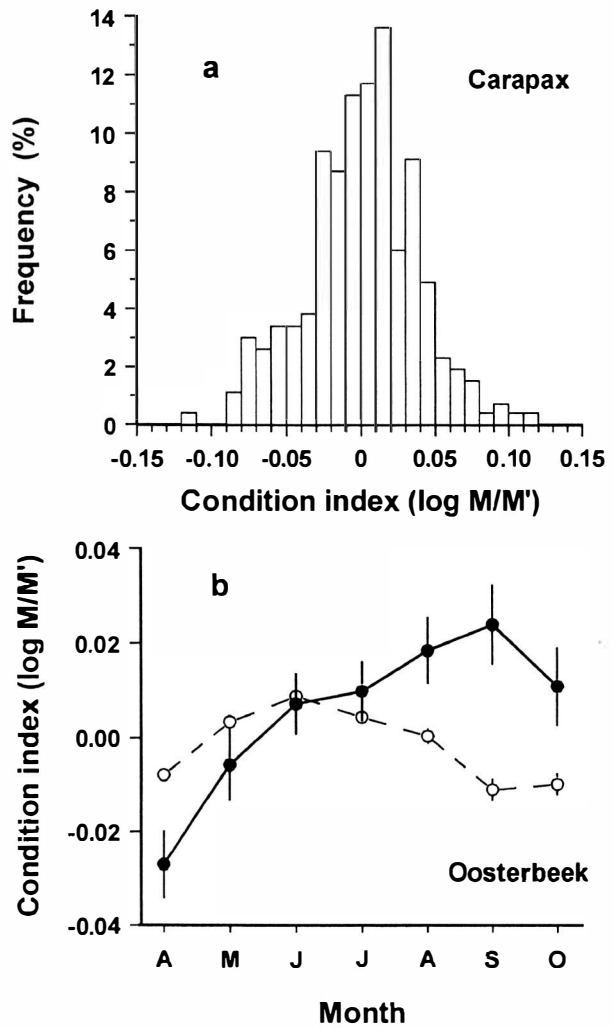


FIG. 1. (a) Frequency distribution of condition index in captive *T. hermanni hermanni* at the Centro Carapax, Italy. (b) Seasonal variation of condition index in *T. hermanni boettgeri*; captive tortoises in Oosterbeek, the Netherlands (solid circles) and wild tortoises in Greece (open circles, from Willemsen & Hailey, 2002). Bars show \pm SE.

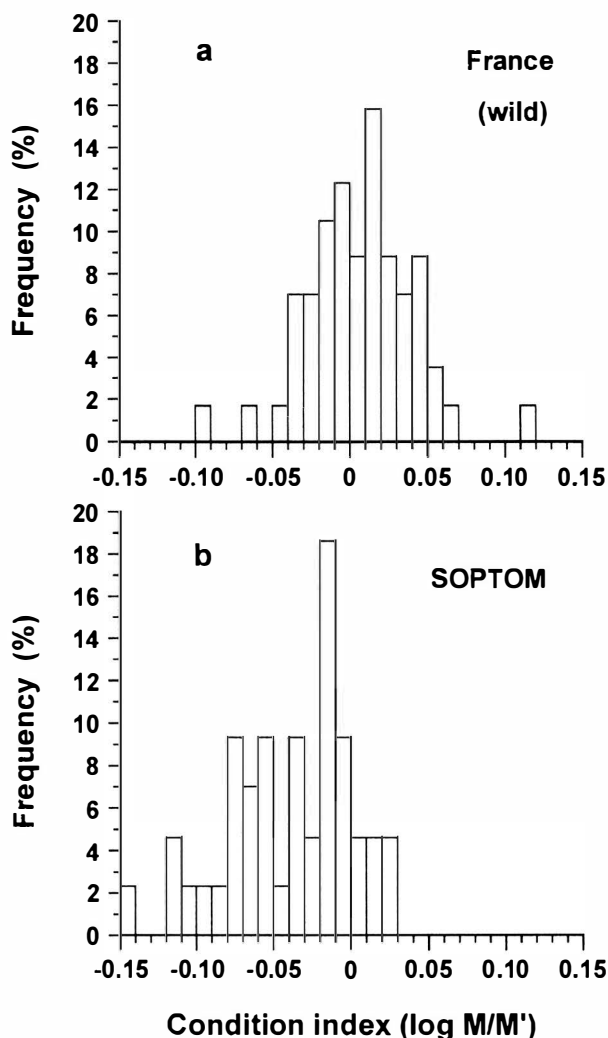


FIG. 2. Frequency distributions of condition index in (a) wild *T. h. hermanni hermanni* in France, (b) captive *T. h. hermanni* at SOPTOM, France.

There were similar results for captive *T. h. boettgeri* at the Centro Carapax, with CI calculated from the mass-length equations for wild *T. h. boettgeri* from Greece. There was no significant difference between females and males ($F_{1,12}=0.494$, $P=0.622$), and the mean was not significantly different from 0 (Table 2). The variability of CI of captive *T. h. boettgeri* at the Centro Carapax was, however, significantly greater than that of the captive *T. h. hermanni* ($\chi^2=10.69$, $df=1$, $P<0.01$). The greater variability may have resulted from the diverse sources and histories of the non-native tortoises at the Centro Carapax, which were acquired as donations and customs seizures, usually of unknown provenance.

The CI of captive *T. graeca* at the Centro Carapax, calculated using the mass-length equations of wild *T. graeca* (Willemsen & Hailey, 2002), varied significantly with sex ($F_{1,22}=7.80$, $P=0.011$). The mean CI was significantly different from 0 in females, which had lower mass than predicted, but not in males (Table 2). The CI of captive *T. marginata* at the Centro Carapax, calculated using the mass-length equations of wild *T. marginata*, did not differ significantly between the sexes

($F_{1,18}=1.45$, $P=0.244$); mean CI was just significantly different from 0 (Table 2), with tortoises having slightly lower mass than predicted.

OOSTERBEEK

The CI of *T. h. boettgeri* in northern Europe was examined in relation to season. A two-way ANOVA of CI with sex and month showed no significant sex \times month interaction ($F_{6,161}=0.21$, $P=0.973$), indicating that the seasonal pattern of CI did not differ between females and males. The seasonal pattern (Fig. 1b) was, however, significantly different from that of wild *T. h. boettgeri* in Greece (Willemsen & Hailey, 2002); two-way ANOVA of CI with status (wild/captive) and month showed a significant interaction of status \times month ($F_{6,6185}=5.82$, $P<0.001$). The CI of the captive tortoises was lower than that of wild *T. h. boettgeri* in April and May, but increased until September, while that of wild tortoises declined after June. Although seasonal patterns of CI differed between wild and captive tortoises, the range of seasonal variation (from about -0.02 to 0.02) was small compared to the total variation. The mean CI at Oosterbeek was not significantly different from 0 (Table 2), and the variability was very similar to that of wild *T. h. boettgeri* in Greece ($SD=0.039$ and 0.038 , respectively).

SOPTOM

The CI of wild *T. h. hermanni* in France was calculated using the mass-length equations for wild *T. h. hermanni* in Italy (above). The CI did not differ significantly between the sexes ($F_{1,55}=3.64$, $P=0.062$). The mean CI was 0.0026 ($n=57$, $SD=0.0437$), not significantly different from 0 ($t=0.44$, $df=56$, $P=0.66$), and most values were between -0.1 and +0.1 (Fig. 2a). The CI of captive *T. h. hermanni* at SOPTOM calculated using the same equations also did not differ between the sexes ($F_{1,41}=0.51$, $P=0.480$). However, the mean CI at SOPTOM was low, and differed significantly from 0 (Table 2) and from that of wild *T. h. hermanni* in France ($F_{1,98}=24.4$, $P<0.001$). The frequency distribution of the CI was shifted towards lower values (Fig. 2b), with 10% of observations being below -0.1 compared to about 1% being below -0.1 in other samples (Fig. 1a; Hailey, 2000; Willemsen & Hailey, 2002). Nevertheless, the variability of the CI at SOPTOM was similar to that of wild *T. h. hermanni* in France ($\chi^2=0.15$, $df=1$, $P>0.05$).

The variability of CI (as measured for example by the SD) is of interest because some putative causes of low mean CI, such as interference competition for food or stress from high aggression, might be expected to increase variation among individuals (with the losers having particularly low values). The variability of CI differed significantly among the seven captive groups described in Table 2 ($\chi^2=20.20$, $df=6$, $P<0.01$). The Oosterbeek sample was the least variable; however, this sample included repeated measurements on the same individuals, which would tend to reduce the variability of

the data. Nevertheless, the variability of CI differed significantly among the remaining six groups ($\chi^2=11.98$, $df=5$, $P<0.05$). The average variability of the CI in all captive tortoises (for comparison with wild populations) was calculated as the weighted (by sample size) mean SD, using the weighted mean variance. The mean SD was 0.044 (calculated from the weighted mean variance).

DISCUSSION

Tortoises in captivity in Italy and in the Netherlands were found to have CI values similar to those in the wild and appeared to be in good health since reproduction was frequent in both centres (R. E. Willemsen, unpublished observations; Eendebak, 1995). Captivity thus does not necessarily lead to a general tendency in *Testudo* (that would be seen in most captive tortoises) to be either underweight or overweight. Spratt (1990) reached a similar conclusion for giant tortoises. Mass-length equations for wild populations may thus be appropriate for calculating the CI of captive tortoises. The western subspecies of *T. hermanni* was slightly heavier than *T. h. boettgeri* at the same length, a difference that corresponds to the more domed shape of *T. h. hermanni* (Ernst & Barbour, 1989). Separate mass-length equations should therefore be used to calculate M' in the two subspecies. Most *T. hermanni* in captivity are *T. h. boettgeri*, of which there was previously a large international trade from the Balkans; relatively few *T. h. hermanni* were exported.

The seasonal pattern of CI of *T. h. boettgeri* at Oosterbeek, with maximum values in late summer, was rather different to that in Greece. This pattern supports the hypothesis that while differences in CI among sites in spring are related to activity and thermoregulation, differences in summer and autumn are related to food availability (Willemsen & Hailey, 2002). The peak of CI was thus late at Oosterbeek, continuing a trend observed from southern to northern Greece. The peak did not correspond to the hottest time of year; temperatures in the Netherlands are maximal in July and August, and decline in September (Anonymous, 1996; data for Winterswijk in the eastern Netherlands). High CI in autumn at Oosterbeek was therefore not due to activity/thermoregulation, but probably to food availability. Mediterranean habitats are characterised by dry summers with low food availability in autumn, and low tortoise activity (Hailey & Willemsen, 2000), whereas food and water were always available in captivity. The results at Oosterbeek show that declining CI in autumn is not constant (for example in preparation for hibernation), but depends on the environment.

Three groups of tortoises had mean CI significantly lower than 0, i.e. body mass on average lower than predicted: *T. marginata* and female *T. graeca* at the Centro Carapax, and *T. h. hermanni* at SOPTOM. The enclosures of *T. graeca* and *T. marginata* at the Centro Carapax were found to have a lack of growing food plants and encroachment of non-edible vegetation. The

low CI of the tortoises prompted increased artificial food supply to those enclosures in May 2000. Changing plant species composition is a potential problem for all captive Mediterranean tortoises in enclosures of natural vegetation. Tortoises consume the available food plants, which are not regenerated quickly enough to provide a sustainable food supply and are replaced by less palatable or less edible species. The enclosures may thus continue to have a green and well-vegetated appearance but contain little natural food.

Scarcity of food plants in enclosures also occurred at SOPTOM, but there was also a problem with respiratory disease (Le Garff, 1998; Pieau, 1999). Both herpesviruses and mycoplasmas have been found in captive tortoises at SOPTOM (Fertard, 1997), although the former were mostly associated with *T. graeca*. The mean CI of *T. h. hermanni* at SOPTOM is equivalent to a relative mass of 91%. A similar finding has been reported in the desert tortoise *Gopherus agassizii*: mass-length relationships differed significantly between healthy tortoises and those with upper respiratory tract disease (caused by *Mycoplasma agassizii*), tortoises with clinical signs weighing on average 7% less than those without (Jacobson *et al.*, 1993). Ten percent of captive *T. h. hermanni* at SOPTOM had CI below -0.1, equivalent to a relative mass of 80%, the recommended threshold for veterinary attention (Hailey, 2000). A report from the Société Herpétologique de France has recommended the separation of *T. h. hermanni* enclosures at SOPTOM from those housing *T. graeca* and *T. marginata* (which may be the sources of exotic diseases in French tortoises), and improved monitoring of health (Pieau, 1999). Measurement of CI would be advisable as part of this monitoring.

An additional problem at SOPTOM was the absence of permanent water points in enclosures. Wild *T. h. hermanni* in France travel large distances to find free water when little succulent plant food is available in summer (Longepierre, 2001). Water availability could affect the CI directly through the degree of body hydration, and indirectly through better ability to digest food when water is available. Extensive studies on American *Gopherus* tortoises show the importance of the interaction between food quality, water availability, and energetics (Medica, Bury & Luckenbach, 1980; Turner, Medica & Lyons, 1984; Nagy & Medica, 1986; Henen, 1997; Nagy, Henen & Vyas, 1998; Wallis, Henen & Nagy, 1999). Drinking allows these tortoises to maintain a positive energy balance when feeding on dry plants in summer. This effect of water on digestibility may have contributed to the low CI at SOPTOM (where most measurements were made in late summer, and water was absent), and to the high CI in late summer at Oosterbeek (where water was always readily available) compared to wild *T. h. boettgeri*.

The variability of CI in captive tortoises (overall $SD=0.044$) was between that of wild *T. hermanni* ($SD=0.038$) and *T. graeca* ($SD=0.039$) and that of wild *T. marginata* ($SD=0.046$) (Willemsen & Hailey, 2002).

There was thus no greatly increased variability of CI in captive populations, even in those groups which had low mean CI (*T. marginata*, female *T. graeca*, and *T. h. hermanni* at SOPTOM). This suggests that captive Mediterranean tortoises do not suffer from aggressive interactions or interference competition for food, which might tend to increase the variability of CI between dominant and subordinate individuals. Such aggressive interactions have often been observed in omnivorous chelonians in captivity (Lardie, 1964; Boice, 1970; Froese & Burghardt, 1974; Boussekey, 1988) and in the wild (Bury & Wolfheim, 1973; Bury, Wolfheim & Luckenbach, 1979; Lovich, 1988). Captive tortoises appear to be less aggressive (Evans & Quaranta, 1949, 1951; Guyot & Lescure, 1994), perhaps because the food of herbivores is less subject to competition in the wild. Aggressive interactions were not observed in captivity during this study.

In conclusion, housing European tortoises in dense groups had no detrimental effect on their body mass condition. Stocking densities ten times greater than the highest population densities in the wild are possible, provided that food and water supplies are adequate and precautions are taken against disease. Further information is needed, however, on the effects of other regimes on the CI and its seasonal variation. Data from non-specialist facilities with fewer tortoises, and from tortoises kept in indoor cages or outdoors in northern Europe without access to glasshouses, would be particularly interesting.

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SHORT NOTES

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SCOLECOPHIDIAN SNAKES IN THE DIETS OF SOUTH ASIAN CAECILIAN AMPHIBIANS

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Sri Lanka, Typhlopidae

A recent review concluded that "Little is known about the diet of caecilians relative to other tetrapods" (O'Reilly, 2000: 148), a deficiency that is symptomatic of many aspects of the biology of the Order Gymnophiona. It is known that caecilians in nature take a variety of invertebrate prey (e.g. Daudin, 1802-3; Wall, 1922; Gliesch, 1929; Taylor, 1968; Largen *et al.*, 1972; Wake, 1980; Gudynas & Williams, 1986; Hebrard *et al.*, 1992; Nussbaum, 1998; Verdade *et al.*, 2000) and that earthworms and termites (soil ecosystem engineers, Lavelle *et al.*, 1998) make up a substantial proportion of the diet of some terrestrial species.

There are fewer reports in the literature of caecilians taking vertebrate prey. Small fish are known to be eaten in the wild by aquatic species such as *Typhlonectes natans* (Fischer) (Lancini, 1969), *T. compressicauda* (Duméril & Bibron) (Moodie, 1978) and *Potomotyphlus kaupii* (Berthold) (Fuhrmann, 1914). Anuran tadpoles have been reported in the diet of *T. compressicauda* (Verdade *et al.*, 2000) and there is a single report of the remains of an adult anuran in the gut of the semi-aquatic *Chthonerpeton indistinctum* (Reinhardt and Lutken) (Prigioni & Langone, 1983). Moll & Smith (1967) reported discovering two lizards (the teiid *Ameiva undulata parva* Barbour & Noble and the polychrotid *Anolis dollfusianus* Bocourt) in the gut of an adult *Dermophis mexicanus* (Duméril & Bibron).

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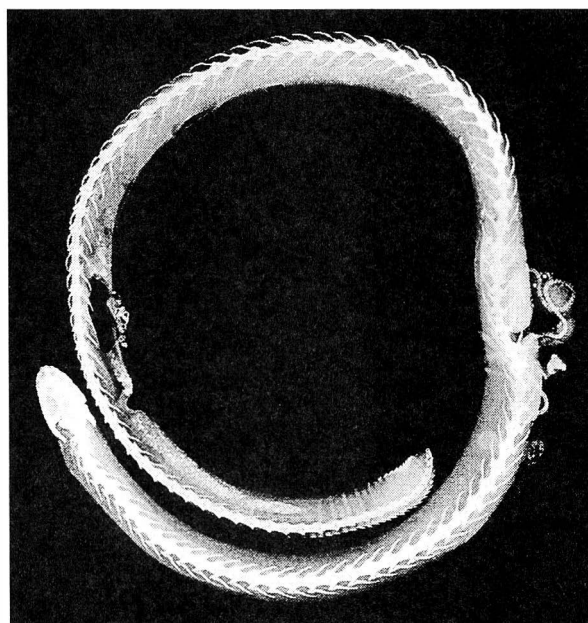


FIG. 1. X-ray from ventral surface of specimen of *Gegeneophis ramaswamii* Taylor showing a scolecophidian snake, *Ramphotyphlops brahminus* (Daudin), inside it. The outline of the snake lies beyond the body of the caecilian because a ventral incision in the body wall resulted in the protrusion of viscera. Total length of the caecilian is 290 mm.

Sarasin & Sarasin (1887-1890) reported that species of *Ichthyophis* eat small burrowing scolecophidian snakes, and Greeff (1884: 18) reported discovering a small scolecophidian in the gut of a *Schistometopum thomense* Barboza du Bocage from the island of Sao Tomé. Here we report and discuss two instances of burrowing scolecophidian snakes in the diets of two south Asian caecilians.

Gegeneophis ramaswamii Taylor is a caeciliid caecilian from the Western Ghats region of southern Kerala, India, where it is common in a wide range of habitats (Oommen *et al.*, 2000). As with many caeciliids, *G. ramaswamii* has a solidly-boned and com-

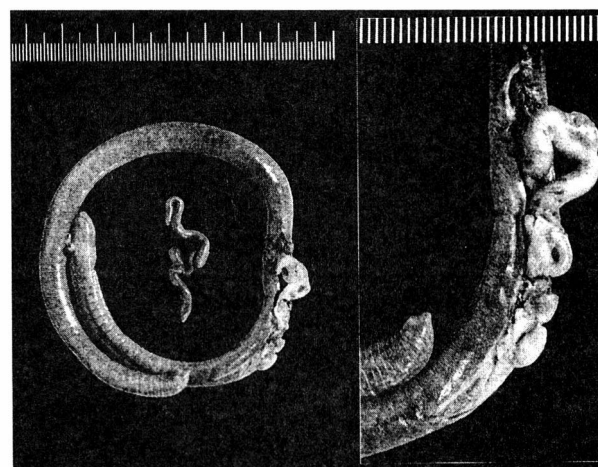


FIG. 2. Photograph of ventral surface of specimen of *Gegeneophis ramaswamii* Taylor and a *Ramphotyphlops brahminus* (Daudin) removed from its gut. Inset shows the snake in the gut prior to dissection. Scale bar in millimetres.

pact skull that lacks an orbit (Ramaswami, 1942) and it is a proficient burrower in soil (pers. obs.). In the course of ongoing studies of this species, a snake was discovered inside one specimen (Department of Zoology, University of Kerala, field tag MW 1292) by X-ray (Fig. 1), and its position within the gut of the caecilian confirmed by dissection (Fig. 2). The *G. ramaswamii* specimen is a relatively large male (total length, TL = 290 mm) collected on 7 July 2000 from near Shonlode, Kanyakumari District, Tamil Nadu (08° 20' N: 77° 23' E, 60 m asl). This is a new locality not reported by Oommen *et al.* (2000), and the second reported presence of *G. ramaswamii* in the state of Tamil Nadu. The specimen was collected from a smallholding (home garden) where it was dug from moist, loose, organically rich soil between a cow shed and a stream. The snake (University of Kerala, MW 2094) is an example of the typhlopoid *Ramphotyphlops brahminus* (Daudin), with a TL of about 85 mm. It had been ingested tail-first and was between one third and one half along the alimentary canal of the caecilian. It was not stretched out, but instead sharply folded along its long axis in several places (Fig. 2). There is very little sign of damage caused by digestion. Other than a small patch near the vent end, the scales are in position and undamaged. Despite being protruded, the tongue is also undamaged. The X-ray reveals no indication of bone damage, though the vertebral column appears to have become partially dislocated toward the middle of the body, where the strongest flexion has occurred.

Ichthyophiid caecilians have a less consolidated arrangement of skull bones than *G. ramaswamii*, and a small, but externally visible eye (e.g. Taylor, 1968). Because *Ichthyophis* species are sometimes encountered in leaf litter and loose soil (e.g. Nussbaum & Gans, 1980), they have been considered surface-cryptic (e.g. Ramaswami, 1941), but they can also be found in firm soil (e.g. Nussbaum & Gans, 1980; pers. obs.). The caecilian fauna of Sri Lanka is currently considered to comprise three endemic species of *Ichthyophis* (Nussbaum & Gans, 1980), with *I. glutinosus* (Linnaeus) being the most widespread on the island. On 18 November 2000, a collection of subterranean vertebrates was made by digging compost and moist soil in a garden adjacent to secondary forest in Tiniyawala near Palawatta, Kalutara District, Western Province, Sri Lanka (N 06° 25' E 80° 19', 175 m asl). Taxa collected included examples of the typhlopoid scolecophidian snake *Ramphotyphlops brahminus* and the caecilian *I. glutinosus*. One living *R. brahminus* specimen (National Museum, Colombo, field tag MW 1768; TL = 122 mm) was temporarily stored in soil in a small plastic bag within a larger bag containing soil and one living *I. glutinosus* from the same locality (National Museum, Colombo, field tag MW 1769; Mass = 30.9 g, TL = 350 mm). In observing these specimens some six hours later, it was found that the snake had escaped from a small hole in the corner of its small bag. It was found within the larger bag, approximately half-protruding, head-first

from the vent of the *I. glutinosus*. The snake was dead, but no significant external damage was apparent. We believe that the most likely explanation for this is that the snake was ingested and egested by the caecilian during the six hours between capture and processing, without being notably digested. An X-ray of the *I. glutinosus* specimen revealed the absence of any other vertebrate prey within the digestive tract.

The single *Ramphotyphlops brahminus* (MW 2049) is the only vertebrate prey found by us in the X-ray examination of approximately four hundred specimens (various institutions) of *Gegeneophis ramaswamii* from many localities in southern Kerala. Similarly, X-rays and dissections (by BP, DJG & MW) of hundreds of ichthyophiids from India, Sri Lanka, and south-east Asia have not previously revealed the presence of vertebrate prey. This suggests that scolecophidian snakes are only a minor part of the diets of these caecilian species. Scolecophidian snakes occur at much lower densities than *G. ramaswamii* and *I. glutinosus* in those habitats in which we have encountered these caecilians (unpublished quantitative data). This is consistent with the hypothesis that these caecilians encounter scolecophidians rarely and feed on them opportunistically.

The generally undigested appearance of the scolecophidian found in the *G. ramaswamii* specimen might be at least partly explained by its relatively anterior position along the alimentary canal. The swift passage of the Sri Lankan scolecophidian through the digestive system of the *I. glutinosus* specimen, and its undamaged appearance, raises the speculative possibility that scolecophidians represent a relatively indigestible class of caecilian prey, from which only limited nutrition can be extracted. It is also possible, perhaps, that the scolecophidian was alive when ingested, and that this, in part, accelerated its rapid passage through the *I. glutinosus* gut. However, there are no background data on the passage time of items through the gut of caecilians.

We recognise that understanding the diets of organisms requires systematic quantitative investigations rather than isolated *ad hoc* reports. However, given the relative lack of data on caecilian diets (O'Reilly, 2000), even isolated reports are worthwhile. Our observations also have additional implications. Gans (1993: 191) suggested that at least Sri Lankan caecilians are generally confined to moist zones: "Consideration of the places where *Ichthyophis* were taken suggests that they always move parallel to streams and do not seem to cross the dry zones between these." Gans contrasted this with the distribution of Sri Lankan scolecophidian snakes (p. 192): "The snakes of the genera *Typhlops* and *Ramphotyphlops* are found on dry surfaces, within dry decaying pieces of wood and beneath rocks." In discussing the significance of fewer probable species of typhlopoid scolecophidian snakes in southern India than in (the much smaller area of) Sri Lanka, Gans (1998: 103) stated that "the relative number of species is clearly the

reverse of that in caecilians. This suggests that the two groups occupy truly distinct subsets of the environment and that its subdivision is highly different." The findings presented here demonstrate that caecilians and scolecophidians (at least the widespread *Ramphotyphlops brahminus*) do interact in nature at least occasionally and that their niches are not absolutely disjunct.

Soil biodiversity is a crucial factor in regulating the functioning of terrestrial ecosystems, but the ecology of soil organisms is relatively little studied and understood (e.g. Copley, 2000). Most studies of soil ecology have focused on non-vertebrate organisms, with the potential importance of the natural vertebrate predators of invertebrate soil ecosystem engineers being largely overlooked. Several groups of reptiles and amphibians are soil-dwelling predators and, in general, we believe their ecology is less well understood than that of their relatives that are found in more conspicuous habitats. Tropical soil communities may include a diversity of vertebrates. For example, at the Tiniyawala locality in Sri Lanka, and on the same day as the *I. glutinosus* and *R. brahminus* were collected, single specimens of the fossorial cylindrophid snake *Cylindrophis maculatus* Linnaeus and of a fossorial scincid lizard *Nessia* sp. Gray were also unearthed. At other Sri Lankan and Indian localities, we have found caecilians in the same habitat as fossorial uropeltid and colubrid snakes. The ecological relations of these co-occurring vertebrates might be expected to be of importance in gaining a fuller understanding of these communities and their impact on soils.

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**FEMALE BREEDING FREQUENCY,
CLUTCH SIZE AND DIETARY
HABITS OF A NIGERIAN
POPULATION OF CALABAR
GROUND PYTHON, *CALABARIA
REINHARDTII***

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Nigeria, reproduction

The Calabar ground python, *Calabaria/Charina reinhardtii* is an Afrotropical burrowing snake, with an unresolved systematic position (e.g. see Kluge, 1993), and is widespread across the Liberia-Congo rainforest belt (Chippaux, 1999). Due to its nocturnal, elusive, and burrowing habits, this snake has been poorly studied (Lehmann, 1971). In recent years, this situation changed rapidly and detailed studies, including radiotelemetric monitoring, have been published utilizing free-ranging specimens from Nigeria (Luiselli & Akani, 1998; Luiselli, Akani & Capizzi, 1998; Angelici *et al.*, 2000). However, the knowledge of some crucial aspects of the ecology of this species, such as reproductive and feeding strategies, is still little known.

In this note, we describe female breeding frequency, clutch size, and dietary habits in a population from a forest-plantation mosaic area of south-eastern Nigeria (see also Luiselli *et al.*, 1998; Angelici *et al.*, 2000). The data were collected between January 1998 and December 2001.

The study site (latitude 04°50'N, longitude 07°59'E; elevation 10 m a.s.l.) consisted of about 30 ha of seasonally flooded secondary swamp-forest almost entirely surrounded by wide plantations, busy roads, and houses, situated in the "Eket" Local Government Area (Akwa-Ibom State). Based on radiotracking data, it is known that these surrounding habitats are generally avoided by ground pythons (Angelici *et al.*, 2000). However, the area was not totally isolated (as demonstrated by the

TABLE 1. Field effort, year-by-year, at the study area. Both the numbers of non-consecutive days spent in the field, and the numbers of yearly consecutive trap-days are indicated.

Year	No. non-consecutive days in the field	No. of consecutive trap-days
1998	41	77
1999	36	89
2000	39	83
2001	43	88

relatively high numbers of "new males" each year; Luiselli *et al.*, unpublished observations), being connected to a nearby forest patch (situated approximately 5 km north of our study site) through a corridor of disturbed bush where ground pythons have been seldom observed.

Since ground pythons are specialized burrowers, they can be captured most easily by pitfalls and/or by removing cover objects, whereas they are rarely found active on the ground (Luiselli & Akani, 1998). Thus, during each study year, three lines of pitfalls connected with drift fences were installed. Each line consisted of 15 pitfalls, placed about 60 m apart, and 100 m from the closest pitfall of the adjacent line. The traps were situated in the same places every year, so that the grid was the same year-by-year. Every snake captured was individually marked by ventral scale clipping, and measured to SVL (to the nearest ± 0.1 cm) and sexed. For females, eventual reproductive status and litter size were determined by abdominal palpation (precision: ± 1 young). Examination of faecal pellets and stomach ingesta allowed us to obtain data on the feeding ecology of the snakes at the study area. The yearly effort in the field, in terms of both number of non-consecutive survey-days in the study area and numbers of days in which traps were placed, is given in Table 1.

All tests, two tailed and with $\alpha = 0.05$, were done using STATISTICA (version 5.0 for Windows) computer package (Statsoft, 1996). When calculating the regression between maternal SVL and number of eggs, to avoid statistical bias due to pseudo-replication of the data (Licht *et al.*, 1966; Hurlbert, 1984), we took body size and clutch size data only once per individual female, i.e. we did not consider further recaptures of the same individual, also if on different years.

In total, we caught 13 adult females and obtained 20 "snake-year" records from females (i.e. counting each female as a separate data point in each year she was collected; Table 2). Reproductive females comprised 11 (=55%) of these records, suggesting an average biennial cycle in individual females. However, this frequency may be biased if reproductive females modify their behaviour (e.g. are active more often above-ground) in such a way that they are easier to find and catch (Bonnet & Naulleau, 1996). So, recapture events of individuals over several consecutive years offer a more reliable indication of female reproductive frequency (Brown, 1991; Capula & Luiselli, 1994; Luiselli, Capula &

TABLE 2. Summary of the longitudinal data collected in the years 1998-2001 from female *Calabaria reinhardtii* at the study area in south-eastern Nigeria. In case of gravid specimens, the number of eggs is presented in parentheses. For the years in which a given specimen was captured, its body size (SVL, cm) is also presented.

Specimen No.	Year 1998	Year 1999	Year 2000	Year 2001
Female 1	81.5 cm (5 eggs)	-	-	-
Female 2	77.5 cm (not gravid)	-	-	-
Female 3	83.1 cm (5 eggs)	83.5 cm (not gravid)	83.5 cm (5 eggs)	-
Female 4	70.2 cm (not gravid)	73.5 cm (not gravid)	-	-
Female 5	78.4 cm (4 eggs)	-	-	-
Female 6	68.8 cm (not gravid)	71.3 cm (not gravid)	-	-
Female 7	-	77.1 cm (3 eggs)	-	-
Female 8	-	88.4 cm (6 eggs)	-	-
Female 9	-	72.7 cm (no gravid)	74.6 cm (3 eggs)	74.7 cm (not gravid)
Female 10	-	-	84.6 cm (6 eggs)	84.6 cm (not gravid)
Female 11	-	-	61.1 cm (no gravid)	63.8 cm (2 eggs)
Female 12	-	-	-	88.6 cm (9 eggs)
Female 13	-	-	-	81.4 cm (5 eggs)

Shine, 1996). We obtained four records of reproductive status of individual females captured in two consecutive years, and two records of reproductive status of females captured in three consecutive years (Table 2). Two females (nos. 4 and 6 in Table 2) were not gravid for two consecutive years (i.e. suggesting an at least triennial cycle), and two (nos. 10 and 11 in Table 2) exhibited reproduction in alternate years (i.e. a reproductive year followed by a non-reproductive year, or vice-versa). In another case, a female from Calabar (Cross River State), was reproductive in two consecutive years (1999 and 2000), suggesting an annual cycle. However, data relative to this individual are not presented in Table 2 as it came from another locality. For females 3 and 9 (in Table 2) we recorded the reproductive condition for three consecutive years. Female no. 3 appeared to exhibit a biennial reproductive cycle, and female no. 9 appeared to exhibit a more-than-annual (perhaps biennial) reproductive cycle. Hence, these data suggest that breeding frequency is likely to be at least biennial.

The females produced 2-9 eggs in their reproductive year, and there was a positive linear correlation between maternal body length and clutch size (Fig 1).

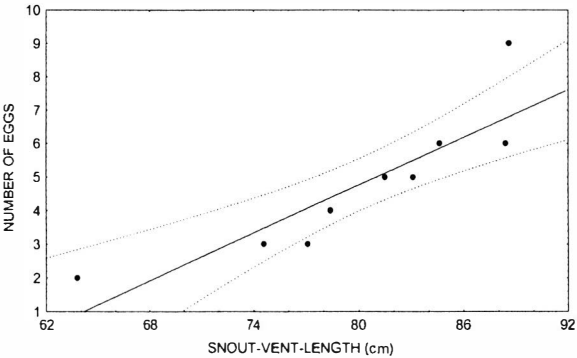


FIG. 1. Relationships between maternal size (cm) and number of eggs in female *Calabaria reinhardtii* from the study area in south-eastern Nigeria. Equation: No. eggs = - 14.25 + 0.237 Snout-vent length; $F_{1,8} = 25.9$, $P=0.0009$.

We examined diet in 44 specimens (16 males, 13 females, and 15 recaptures). We collected faecal pellets from 16 snakes with identifiable prey remains, and obtained stomach ingesta from seven specimens. Regurgitated items were adult *Mus musculoides* in two cases; young *Rattus rattus* in two cases; nestling mice (of unknown species) in two cases; and snake eggs (probably of *Psammophis phillipsi*) found in one specimen. Faecal pellets included exclusively rodents (faeces from 10 specimens: *Rattus* sp. in four; *Mus musculoides* in four; undetermined species in two) and shrews (most probably of the genus *Crociodura*, found in faeces of six specimens). Interestingly, shrews were absent from the diet of all pythons that had consumed rodents. In any case, the diet of *C. reinhardtii* at the study area proved to consist almost only of small mammals, apart from the single case of snake eggs in the stomach.

Although very detailed longitudinal data are available for some species of tropical Booidea (e.g. Madsen & Shine, 1996, 1998, 1999a,b, 2000; Shine & Madsen, 1997; Shine *et al.*, 1997), we cannot make sound comparisons between our data and those presented in such studies because (1) our sample size is too small compared to Madsen & Shine's studies; (2) *Calabaria* has a still unresolved systematic position; and (3) it has specialized burrowing habits that may have produced very specific life-history adaptations for life underground, at least if compared with typical terrestrial-arboreal boids. So, it would be better to compare our data with those relative to other subterranean boids, such as *Charina bottae* (cf. Hoyer & Stewart, 2000a,b), which has been considered a species phylogenetically closely related to *Calabaria* (Kluge, 1993). However, it must be stressed that our study area in tropical Africa is climatically very different from temperate North-America, where *C. bottae* is living, so that these comparisons may also have considerable biases due to inter-climatic differences. However, after having taken into account these potential biases, it should be stressed that both the main reproduc-

tive characteristics and the diets of Calabar ground pythons were similar to that of *C. bottae*. Indeed, Hoyer & Stewart (2000a) reported a biennial reproductive frequency and an average litter size of 2-5 in *C. bottae* from California, which is similar to our population of *C. reinhardtii*. In addition, small mammals were also found to be the major prey items in *C. bottae* (Hoyer & Stewart, 2000b), although this latter preyed frequently upon reptile eggs (lizards and snakes), which were consumed only occasionally in our *Calabaria* population. This difference is noteworthy and probably reflects true ecological differences between species, as at least four species of oviparous snakes (*Python regius*, *Naja nigricollis*, *Psammophis phillipsi*, and *Lamprophis lineatus*) and one species of appropriately sized lizard (*Agama agama*) are common in the study area, and may provide an abundant food resource for *Calabaria* specimens. In any case, the data presented here, together with those already presented by Luiselli *et al.* (1998) and by Luiselli & Akani (1998), confirm that *C. reinhardtii* is primarily a small mammal-eater, and does not feed upon earthworms (as observed in captive specimens, cf. Trutnau, 1981). One *Mabuya* skink was, however, found in the stomach of another Nigerian individual (Luiselli & Akani, 1998).

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BODY TEMPERATURES OF TWO VIVIPAROUS *LIOLAEMUS* LIZARD SPECIES, IN PATAGONIAN RAIN FOREST AND STEPPE

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Key words: body temperature, *Liolaemus*, lizard, reproduction, thermoregulation

The genus *Liolaemus*, with at least 160 known species, is broadly distributed from the highlands of Peru and Bolivia to Tierra del Fuego, and from the Pacific islands of Chile to coastal Brazil (Cei, 1986). *Liolaemus elongatus* and *Liolaemus pictus* are distributed along an east-west precipitation gradient from the Patagonian steppe to the rainforest and they are sympatric in the transition zone located along the east of the Andes. *Liolaemus elongatus* is a saxicolous, viviparous, medium- to large-sized lizard (23 - 90 mm snout-vent length, adults and juveniles included). This species is distributed from San Juan to Chubut and is associated with steppe environments (Cei, 1986), but it has been found in the rainforest-steppe transition, in a very humid, cloudy microhabitat, syntopic with *Liolaemus pictus* (Ibargüengoytía & Cussac, 1998). *Liolaemus pictus* is a viviparous, medium-sized lizard (23-75 mm snout-vent length, adults and juveniles included) that lives in temperate habitats from sea level to moderate elevations (1600 m), in leaf-litter and under logs (Donoso-Barros, 1966).

We have shown previously that *L. elongatus* has an annual or biennial female reproductive cycle, while *L. pictus* has a biennial or triennial female reproductive cycle. As there is a strong association between the rate of embryonic development and temperature (Heulin, Osenegg & Lebouvier, 1991; Gilbert, 1994; Andrews, Mathies, Qualls & Qualls, 1999), we suggested that the low frequency of reproduction observed in these species is related to a short, cold, active season which constrains opportunities for thermoregulation, in turn affecting vitellogenesis and embryonic development (Ibargüengoytía & Cussac, 1996; 1998).

In the present work, we report the body temperatures at capture of lizards from two sympatric populations of *L. elongatus* and *L. pictus* in a rainforest site, and of a steppe population of *L. elongatus* alone. In an effort to

understand the relationship between environmental temperature and reproduction, the objectives of this preliminary study were to determine (1) the general thermobiology characteristics of the two species; (2) whether there are intraspecific differences between a steppe and a rainforest population of *L. elongatus*; and (3) whether reproductive status is related to body temperature in *L. elongatus*.

Measurements were made on *L. pictus* (eight lizards) and *L. elongatus* (16 lizards) from sympatric populations occupying a rocky promontory on the eastern shore of lake Moreno (rainforest site) near San Carlos de Bariloche, Río Negro, Argentina (41° 10' S; 71° 30' W, 700 m above sea level), from September 1996 to April 1997. Another *L. elongatus* population was studied in the steppe (53 lizards) in San Carlos de Bariloche (41° 6' S, 71° 7' W, 800 m above sea level), from September 1997 to February 1998 and from October 2000 to February 2001. Animals were caught by noose, toe clipped for identification upon recapture, and subsequently released.

The cloacal temperature (CT) was considered equivalent to body temperature (*sensu* Pough & Gans, 1982) and was measured using a catheter probe (TES TP-K01, 1.62 mm diameter) introduced about 1 cm inside the cloaca. The animals were handled by the legs to minimize heat transfer and temperature was recorded within 20 sec. of handling.

The temperatures of active and non-active individuals were measured, the latter caught only in the steppe site. Active individuals were defined as lizards found outside the burrows, performing any behaviour related to feeding and/or breeding activities. Non-active individuals were captured inside rock crevices and their temperatures show the thermal consequences of avoiding predation risk and/or the absence of adequate environmental conditions for activity.

We recorded the date and time of collection, snout-vent length (SVL, using a vernier calliper) and microenvironmental temperatures. The temperature of the substratum (T_s) was measured using a TES TP-K03 probe; air temperature (T_a) was measured 1 cm above the ground, in the case of lizards in activity, or inside the crevice, in the case of non-active individuals, using a TES TP-K02 gas probe. Thermocouples were connected to a TES 1302 thermometer (TES Electrical Electronic Corp., Taipei, Taiwan).

Maximum juvenile SVL was taken as 54 mm for *L. elongatus* and 49 mm for *L. pictus*, following Ibargüengoytía & Cussac (1996; 1998). Sex was determined in both species from the precloacal pores which are present only in males (Cei, 1986). Lizards were assigned to one of four reproductive categories: (1) juveniles; (2) adult males; (3) adult, non-pregnant females; and (4) pregnant females (recognized by their swollen body; Smith, 1997). The swollen body can be recognized from the beginning of pregnancy as these species are mainly lecithotrophic and the recently-ovulated oocytes are similar in size to the fetuses in advanced pregnancy (Crocco, 2001).

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TABLE 1. Cloacal temperature (T_c) and environmental temperatures (T_a and T_s) of *L. elongatus* and *L. pictus* from the rain forest and steppe sites. * indicates the significance of either paired Student's *t*-test or Wilcoxon Signed Ranks Test ($P < 0.01$).

Species	Habitat	Cloacal temperature (T _c)	N	Environmental temperatures	T _c vs T _a or T _s
<i>Active lizards:</i>					
<i>L. elongatus</i>	Rain forest	33.2	29	T _a =23.3	Z=4.63*
			29	T _s =27.7	Z=4.18*
<i>L. elongatus</i>	Steppe	29.8	40	T _a =20.0	Z=5.22*
			40	T _s =25.3	Z=3.42*
<i>L. pictus</i>	Rain forest	33.2	8	T _a =24.6	Z=2.53*
			7	T _s =30.2	Z=1.35
<i>Inactive lizards:</i>					
<i>L. elongatus</i>	Steppe	11.7	18	T _a =11.9	t=1.0
			17	T _s =11.3	t=0.5

Regression analysis, paired Student *t*-test, and one-way analysis of variance (ANOVA), were used in the analyses. Assumptions of normality and homogeneity of variance were tested using the Kolmogorov-Smirnov test and Levene's test, respectively. When parametric assumptions were not met, non-parametric Spearman's correlation coefficient, Mann-Whitney Rank Sum Test (MW) and Kruskal-Wallis H (KW) tests were used.

Cloacal temperature in *L. elongatus* from the rainforest was not significantly correlated with SVL ($r_s=0.09$, $n=27$, $P>0.62$), but was positively and significantly correlated with both T_s and T_a ($r_{s(Ts)}=0.60$, $r_{s(Ta)}=0.54$, $n=29$, $P<0.001$). Cloacal temperature was, on average, significantly higher than both T_a and T_s (Table 1, Fig. 1).

Cloacal temperature of active, thermoregulating *L. elongatus* from the steppe site showed a negative rela-

tionship with SVL ($r_s=-0.28$, $n=69$, $P<0.01$) and a positive relationship with both T_s and T_a ($r_{s(Ts)}=0.49$, $r_{s(Ta)}=0.44$, $n=69$, $P<0.001$). The cloacal temperature was significantly higher than the environmental temperatures (Table 1, Fig. 1). Cloacal temperatures of non-active *L. elongatus* from the steppe site showed a significant relationship with T_s and T_a (regression, $F_{2,14}=33.08$, $P<0.001$, Fig. 1) but they did not show a significant relationship with SVL ($P>0.9$). Cloacal temperature of non-active *L. elongatus* did not differ either from T_a or from T_s (Table 1).

Liolaemus pictus cloacal temperature was not significantly related to SVL, T_s or T_a ($r_{s(SVL)}=0.38$, $r_{s(Ts)}=0.07$, $r_{s(Ta)}=0.54$, $n=7$, $P>0.3$). However, cloacal temperature was significantly greater than T_a (Table 1, Fig. 1).

Data from *L. elongatus* showed no significant difference among sex and reproductive categories, either in the rain forest (KW: $H_{3,17}=2.09$, $P>0.55$), or in actively thermoregulating (KW: $H_{2,54}=2.54$, $P>0.28$) or non-active *L. elongatus* from the steppe (ANOVA: $F_{1,15}=1.161$, $P>0.29$). Nevertheless, there was a tendency for pregnant females to have somewhat lower cloacal temperatures (Fig. 2).

Cloacal temperatures of *L. elongatus* from the steppe site were significantly lower than those of the same species from the rain forest (ANOVA: $F_{1,68}=6.46$, $P<0.013$), taking into account T_s as a significant covariable in the model ($P<0.001$). Residuals obtained from the regression of cloacal temperature versus T_s were calculated and compared in Fig. 2.

The data show that *L. elongatus* and *L. pictus* body temperatures in activity were higher than environmental temperatures up to approximately 35 °C, beyond which body temperature no longer increased. Body temperatures which are higher than microhabitat temperatures are also known in other lizard species, such as *Liolaemus multiformis* (Pearson & Bradford, 1976), *Kentropyx calcarata* (Vitt, 1991), *Ameiva ameiva* (Vitt & Colli, 1994) and *Sceloporus jarrovi* (Smith & Ballinger, 1994a). The relationships between body and environ-

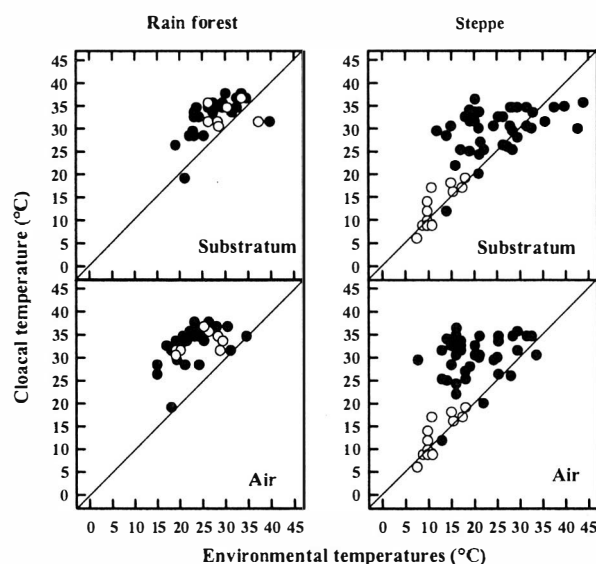


FIG. 1. Cloacal temperature versus the environmental temperatures; substratum temperature (upper panels), air temperature (lower panels). *Liolaemus elongatus* (filled circles) and *L. pictus* (open circles) from the rain forest (left panels) and active (filled circles) and non active (open circles) *L. elongatus* from the steppe site (right panels).

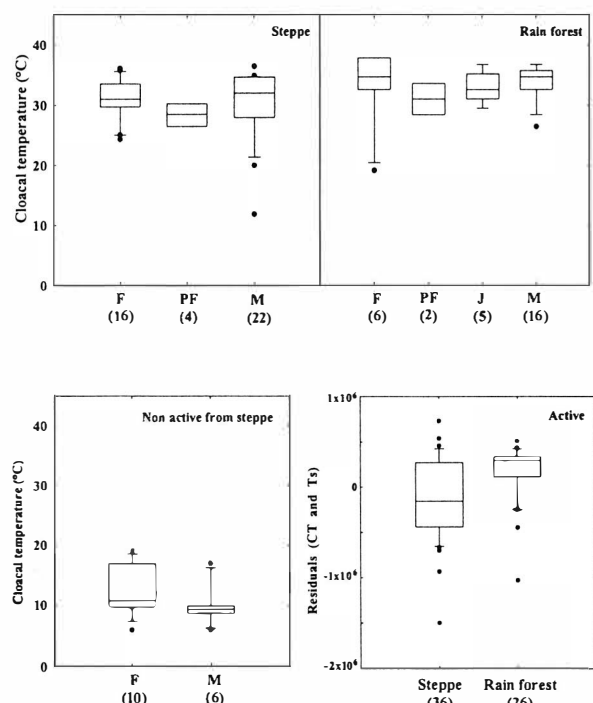


FIG. 2. Cloacal temperatures at capture (CT) of active *L. elongatus* from steppe and rain forest (top), cloacal temperature of male and female non-active individuals from steppe (lower left), and cloacal temperature (residuals of the regression versus substratum temperature, T_s) from steppe and rain forest active individuals (lower right). F: females, PF: pregnant females, J: juveniles, M: males. Median, quartiles and data outside 10th and 90th percentiles are indicated. In brackets are the numbers of observations.

mental temperatures of *L. elongatus*, particularly in the steppe, indicate thermoregulation abilities. Although an individual was captured basking with a cloacal temperature of 11 °C, most of the lizards with body temperature lower than or equal to 20 °C were found in their burrows. This result probably reflects a severe constraint imposed by predation risk.

Although annual means of minimum, maximum and daily mean temperatures for Patagonia show that heat availability is higher in the steppe than in the rainforest (Correa, 1998), we recorded lower cloacal temperatures of *L. elongatus* from the steppe, even when the effect of substrate temperature was removed from the analyses. This difference may be attributable to differences in habit in the two environments, and reinforces the hypothesis of plasticity in the thermo-responses of *L. elongatus*.

The most commonly cited immediate benefit of viviparity in reptiles is that pregnant females can hasten embryonic development via thermoregulation and partially compensate for a short active season (Shine, 1985). Accordingly, it has been predicted that pregnant females should select higher and/or less variable body temperature than would non-pregnant females and adult males (Charland & Gregory, 1990). One postulated benefit of moderating body temperature during pregnancy is to enhance embryo survival, since high incubation temperatures are detrimental to embryonic development (Mathies & Andrews, 1997). We failed to find signifi-

cant differences among sex categories in cloacal temperature of *L. elongatus* in any of the localities. Nevertheless, there was a tendency for pregnant females to show lower cloacal temperatures than the other individuals. A similar pattern was observed in the viviparous lizard *Sceloporus jarrovi* (Smith & Ballinger, 1994a) and in the oviparous *Sceloporus virgatus* (Smith & Ballinger, 1994b), and was attributed to different microhabitat use in order to avoid the predation risk associated with carrying embryos or eggs and to maximize developmental success (Smith & Ballinger, 1994a).

In the light of our results we propose that under the restrictive environmental conditions of Patagonia, major differences arise between the body temperatures of active and non-active individuals. The former are able to thermoregulate, and pregnant females show a (non-significant) tendency to maintain this stability by choosing lower temperatures and, in consequence, lengthening the pregnancy. If this hypothesis could be confirmed, a causal link for the low female reproduction frequency observed in *L. elongatus* and *L. pictus* would be established. Further studies are necessary to advance in the thermobiology of these species.

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BOOK REVIEWS

The Cane Toad. The History and Ecology of a Successful Colonist. Christopher Lever (2001). 230 pp. Westbury Publishing, West Yorkshire £44.00 (cloth).

The mere mention of the name "Cane Toad" inspires fear and loathing in the hearts of many Australians such as myself. The tales of treachery and tyranny associated with this humble amphibian are legendary. As a child in Fiji I grew up with these curious "Botos" (as the Fijians called them), watching them appear in their dozens on the lawn on a balmy tropical evening. I also observed with morbid fascination, their crispy dried carcasses that covered the roads, all victims of some high speed driving. As a child, I wasn't aware of the cane toad's negative effect on its ecosystem. Nowadays most Australians are well versed in the cane toad story. In a bid to make the best of a bad situation, one enterprising Queenslander has even developed a range of dried cane toads in all sorts of comical poses. It is perhaps because the cane toad story is so well known, that I have never read an entire book devoted to them before. This book by Christopher Lever certainly provides all the information and insights you could ever need on the creature we all love to hate.

This book provides a thorough, comprehensive and scientifically sound treatment of all aspects of the cane toad and its biology. Lever starts with a general introduction on invasive species, including the motives and consequences of introductions, various aspects of introduced species biology, concluding with the impact of naturalized animals and biological control. The first chapter is a review of the natural history of the species. This involves a detailed summary of nomenclature, natural distribution, habitat, systematics, description, vocalization, feeding habits and so forth. Information in this section is concise and exhaustively researched, providing the latest references in many cases. Of particular and novel interest was a section describing the diseases, parasites, fungi and toxins that have been known to affect the cane toad. This detailed outline will be of great interest for those researchers plotting ways of halting the spread of this species. Of additional novel interest is a section detailing the use of cane toads in human pregnancy testing!

The next chapter provides an exhaustive, and I feel, long-winded, summary of the entire current range of the species. The treatment is well-researched and thorough but occupies nearly half of the book (105 pages). The information will be of interest to readers who wish to research all the information known about the cane toad in their particular country. It contains blow-by-blow accounts of the introduction history of the species in that country and their eventual spread or demise. In a book attempting to provide a thorough treatment of the species and its naturalization, this chapter has its place and

is very detailed. I just found it a little verbose. Also on this point, I presumed the book was about Australia (although this wasn't mentioned explicitly) in which case all the other detailed country information is not necessary. To add support to this, the remaining chapters deal in detail with Australia and not any other country.

The third chapter details the impact of the cane toad in Australia. I felt that this was the real substance of the book and provided the most interesting information on the economic, social and environmental impact of the species in Australia. Of particular interest to myself as an ecologist, were the accounts of the impacts of cane toads on native predators as well as the decline of native frogs within the range of the cane toad. The next chapter follows on well from this, describing the current knowledge and advances in biological control of this species.

Chapter five titled "Evolutionary Experiments" appears to be a bit of a throwaway chapter in that it is only two pages long. It is however a useful treatment of the presumably few studies that have used introduced populations for studies of speciation, genetic drift and bottlenecks.

The last chapter is perhaps the one that I have the greatest problem with. It is titled "Declining Amphibian Populations". Now although I am an amphibian conservation biologist and supporter of the DAPTF, I have real trouble in seeing how this chapter meshes with the book. The chapter is undoubtedly a well-researched and timely warning on the declining amphibian problem. I would have expected, however, that perhaps it might include information on the impacts of the cane toad on native frogs, and the role of cane toads in declines. However, there is scarcely a mention of the cane toad in this chapter. It would appear that this chapter has simply been tacked on, in the current and popular climate of amphibian declines, rather than having a great deal to do with the topic of the book. The eventual thesis in the closing sentence of this chapter, is that research on amphibian declines may result in discovering a potential biological control agent for the cane toad. I just can't help feeling that so much more could have been done with this chapter.

Overall, this is a thoroughly researched and well-presented book with well-produced maps and 11 colour figures. Unfortunately the book fell short of its mark in a few places and seemed to suffer from trying to present too much at once. However, I would highly recommend the book to researchers and students alike. The author has undertaken an enormous amount of thorough research to produce an accurate and insightful book. The price is hefty but this work is a major contribution to its field and will likely stand alone as an important reference for many years to come.

Robert A. Davis

University of Western Australia

The Physiological Ecology of Vertebrates. A View from Energetics. (2002) 576 pp. Brian Keith McNab. Cornell University Press, Ithaca, New York. US\$48.95 (cloth)

Physiological ecology is the study of the mechanisms in their environmental and/or evolutionary context. But as the author rightly points out these contexts are not always intelligible for any particular organism. The author's solution to this difficulty is to restrict the discussion to vertebrates and to use a common theme to facilitate explanations – this theme is energetics. This is a sensible approach as energy availability and use are demonstrably crucial elements for the individual, group, community, or even larger units. The author's methodology is an inductive one, searching for correlations and explanations *a posteriori*, an approach that often shifts the focus from the general to the unusual example. This contrasts with the theoretical approach, which, the author opines, is '...symptomatic of the times, when the search for theory in ecology outweighs a commitment to collection of data'. The author is unapologetic for his views, arguing cogently that his methodology is of great utility in formulating hypotheses for future research. Consequently, the book is replete with detailed, yet pertinent examples, yet is still a good read with 'boxed essays' containing the author's more partisan views on a variety of topics. The book is intended to be an essay, which indeed it is, and well-written one.

In his research area Brian McNab has a reputation for unfashionable views forthrightly promulgated. This is to his credit, as science needs such debate for its health. However, here it has an unfortunate consequence – repetition. Not every reader will be as involved as the author in the debate on the relevance of phylogenetic contrasts to comparative studies. We are introduced to this particular controversy by page 5 and it reappears in 'boxed essays' on pages 100 and 440. Yes, this is important; however, a neutral reader may not feel quite so passionately as the author evidently does!

Although, organised into five parts the essay actually starts with the Preface, continues in an Introduction (both are essential reading) and the arguments are developed in the first chapter on 'Limits to Adaptation'. The first part, 'Foundations', focuses on thermal exchange (the author's principal speciality) and on the principles of osmotic and gaseous exchange. Part 2 contains around 100 pages on thermal exchange with the environment and includes a review of the scaling of metabolism, as well as considering the adaptations of ectotherms and endotherms. Part 3, 'Material Exchange with the Environment' covers osmotic exchange, water and salt fluxes, as well as adaptation of gas exchange. There follows a lengthy section on ecological energetics dealing with energetics of locomotion, energy budgets, periodicity in the environment, as well as diet and nutrition (over 150 pages).

Some of these threads are woven together in the final part 'Consequences'. Although short (30 pages) this discussion on the interaction between physiology and geographic distribution is perhaps the most thought provoking, with valuable introductory synopses on gestation period, generation time, fecundity, postnatal growth and life span. The consequences of energetics for geographic distribution include a number of herpetological case histories: sea snakes, American alligator and the terrestrial ectotherms of North America; osmotic limits for plethodontids, crocodiles and alligators; the impact of climate change on montane amphibians. Throughout the text the adaptations exemplified are probed and discussed, frequently ending with the formulation of unanswered questions. All chapters have enumerated summaries of the main conclusions.

What is there in this book for the herpetologist? Most chapters are organised taxonomically, so that information on amphibians and reptiles is easy to find, although sometimes they are lumped with other ectotherms. For example, the author discusses behavioural temperature regulation in terrestrial environments, physiological control of heat exchange in reptiles (and its evolution), thermal relations of dinosaurs, as well as the advantages of ectothermy. In the section on osmotic regulation, amphibians are considered both as aquatic (osmotic regulation in anurans) and terrestrial vertebrates (nitrogenous end products and desiccation), and there is detailed coverage of renal and extrarenal excretion in reptiles. There is a thorough consideration of bimodal gas exchange in amphibians, lungless salamanders, and the reasons and consequences of a return to aquatic exchange in reptiles. Amphibian and reptilian locomotion features in the section on energetics of locomotion, but there is rather less on ecological energetics (budgets) except for consideration of hibernation and aestivation in ectotherms; there is very little herpetological in the chapter on diet and nutrition.

However, this book is not encyclopaedic. For example, there is little consideration of the physiological ecology of the avian embryo, for which there is an extensive body of recent literature. Likewise, the review of the energetics of avian incubation is partial, focused on egg neglect in seabirds, mound building in megapodes, and penguin incubation, even though there is an abundant recent literature. This illustrates the main problem with this book. Whilst there are more than 3100 references this text is essentially a retrospective. As a review of research from the 60's, 70's and 80's it is exceptional; for example, the summary of Victor Hutchinson's studies on gas exchange in amphibians is invaluable. But, as for any book attempting to be comprehensive, there will be areas less familiar to the author. The retrospective nature of this text makes these sections much the weakest. That said, recommend, persuade or perhaps even cajole your graduate students to read this text, even if it isn't their field. For if they are to pursue a career in biology,

then they too should be able to enthuse and communicate their passion for their subject after almost 40 years of research – just as Brian McNab has done so brilliantly in this text.

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Amphibians and Reptiles of Pennsylvania and the Northeast. Arthur C. Hulse, C. J. McCoy & Ellen J. Censky. (2001). 430 pp. Cornell University Press, Ithaca, New York. £26.95 (cloth).

As the two-page Preface explains, this volume was inspired by that of 1982 on amphibians and reptiles in Pennsylvania by the late Jack McCoy, to whom – along with Caleb Hulse, son of Arthur – the present book is dedicated. Originally intended as an account of the natural history of Pennsylvania's herpetofauna, it was later decided that the scope be extended to cover the entire north-eastern United States. The Acknowledgments (one page) indicate that the 42 very satisfactory figures are by Linda Witt Fries.

The 36 pages of the Introduction provide background information on landform, climate and vegetation, as well as providing an explanation of the species accounts to follow, notes on observing and collecting, and valuable identification keys to all the species covered in the next two chapters – on Amphibia (150 pages) and Rep-

tilia (173 pages). The latter consist of detailed species accounts and include 83 maps which reflect the greater distributional emphasis given to Pennsylvania itself. The 133 colour photographs in 16 plates are mostly good, although some are a little small and there are occasional errors – such as the use of the scientific name *Rana utricularia* for the southern leopard frog, rather than *R. sphenoccephala*, used elsewhere in the text.

The Appendix gives mensural and reproductive data for most of the Pennsylvania species in its 15 pages, there is a five page Glossary, some 600 sources are listed in the 32 pages of Literature Cited, and the five-page Index lists the animals featured in the preceding chapters.

Intended for a wide readership, from the interested rambler to the professional herpetologist, this book will be of considerable interest to all and of great value to many. The authors have been honest about gaps in knowledge and hope that readers will be encouraged to further investigate these themselves. Information on US species is generally accurate, although there are some mistakes in metric/imperial conversions, typographical errors, and poor or incorrect punctuation – sometimes due to little other than commas and full stops being used for several pages; also, the map for one species often appears within the text for the next. These are minor criticisms, however, and do not prevent me from recommending this book.

Leigh Gillett
British Herpetological Society

ERRATUM

Akani, G. C., Eniang, E. A., Ekpo, I. J., Angelici, F. M. & Luiselli, L. (2002). Thermal and reproductive ecology of the snake *Psammophis phillipsi* from the rainforest region of southern Nigeria. *Herpetological Journal* **12**, 63-67.

Figures 3 and 4: The caption for Fig. 3 relates to Fig. 4, and vice versa.

Page 64, Results, Reproductive Cycle, line 8: For "March ($n=11$)" read "May ($n=11$)".

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Dunson, W. A. (1969a). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

Dunson, W. A. (1969b). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* **216**, 995–1002.
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