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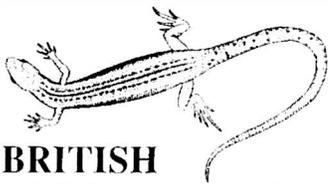
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FRONT COVER: *Caiman crocodilus* (B. Lewis)

## DO MOUNTING VOCALISATIONS IN TORTOISES HAVE A COMMUNICATION FUNCTION? A COMPARATIVE ANALYSIS

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We review the occurrence of vocalisations in tortoise courtship in order to investigate their functions, if any, taking into account evolutionary history, habitat and body size. Courtship behaviour has been described for 101 (41%) of all chelonian species. Among them, mount vocalisations occur in 35 species belonging to families Testudinidae Batsch, 1788 ( $n=29$ ), Trionychidae Fitzinger, 1826 ( $n=3$ ), Emydidae Rafinesque, 1815 ( $n=2$ ), and Bataguridae = Geoemydidae Theobald, 1868 (including Batagurinae Gray, 1869) ( $n=1$ ). The mapping of vocalisation evolution along the phylogenetic tree revealed that mount vocalisations are an ancestral trait, being present from the origin of Cryptodira, and calls mainly occur in terrestrial or semi-terrestrial species. In the species and subspecies of Testudinidae we considered, mounting-calls show an harmonic structure with frequency and amplitude modulation, which are acoustic features not compatible with mechanisms of sound production based simply on air flow through respiratory tracts. Moreover, the call fundamental frequency was negatively related to body size. Since in birds and amphibians such size-frequency relationship is due to a correlation existing between body size and mass of the vibrating device used to produce sounds (a greater device produces longer wavelengths and consequently low frequencies), in tortoises vocalisations might also be produced by vibrating structures not yet described. All these findings strongly suggest that mounting-calls might have the function to convey information on the signaller to conspecifics, possibly influencing the outcome of sexual interactions.

*Key words:* body size, call frequency, chelonians, mount vocalisations, phylogeny

### INTRODUCTION

The courtship and copulatory behaviour of the chelonia is elaborate, and based on a multiple signalling system involving visual, olfactory, and acoustic signals. Particularly interesting are the vocalisations associated with mounting, as this is the predominant – or for some species the only – behaviour during which turtles vocalise (Ernst & Barbour, 1989). Despite this, chelonian vocalisations have been studied only occasionally (Campbell & Evans, 1967, 1972; Mrosovsky, 1972; see also Gans & Maderson, 1973 for a review), and almost nothing is known about their production and function (Berry & Shine, 1980; Swingland & Stubbs, 1985; Olsson & Madsen, 1998). The lack of interest in the acoustic behaviour of chelonians may have resulted from the general belief among herpetologists that turtles and tortoises lack a functional sense of hearing (e.g. Pope, 1955), but at present we know from many studies that a number of species have a considerable auditory sensitivity to sounds below 1000 Hz (Wever & Vernon, 1956a,b; Gulick & Zwick, 1966; Patterson, 1966; Campbell & Evans, 1967; Ridgway *et al.* 1969), and this no doubt enables the animal to perceive many acoustic signals both on land and in water. Therefore, the expla-

nation proposed by many authors that tortoise vocalisations are simple “noises” involuntarily produced by copulatory movements (Weaver, 1970; Mrosovsky, 1972) sounds very unlikely. In a recent study on *Testudo marginata* Schoepff, 1793 (Sacchi *et al.* 2003), we showed that two features (call rate and duration) of male calls strongly correlated with male mounting success (number of effected mounts and number of mounted females by a male). Also, in *Testudo hermanni* Gmelin, 1789 we found that different call features (i.e. calling rate, frequency range, call duration) were significantly related to various aspects of male quality (weight and body size, general health condition, and mounting success, P. Galeotti & R. Sacchi unpublished data), suggesting a possible adaptive role of vocalisations in conveying information about male general quality in socio-sexual contexts.

In addition, we found that call fundamental frequency of both species was negatively related to male body size (weight, head and carapace length). This last finding suggests that sound production in tortoises might be due to a specific vibrating structure, since in both leptodactylid frogs and birds, which use vocal cords and the syrinx for generating sounds, the fundamental frequency of vocalisations similarly decreases with increasing body size (Ryan, 1985; Ryan & Brenowitz, 1985). In fact, body size affects sounds by determining the size of vibrating structures producing them, and animals can most effectively produce sounds

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TABLE 1. Number of males and acoustic features of calls (mean  $\pm$  SE) for the species of Testudinidae recorded.

Species	No. individuals	No. vocalizations	Duration (ms)	Gap between calls (s)	Fundamental frequency (Hz)
<i>Geochelone chilensis</i> = <i>Chelonoidis chilensis</i> (Gray, 1870)	1	12	278 $\pm$ 23	2.15 $\pm$ 0.12	209.2 $\pm$ 2.1
<i>Geochelone carbonaria</i> = <i>Chelonoidis carbonaria</i> (Spix, 1824)	2	52	84 $\pm$ 2	0.29 $\pm$ 0.02	109.9 $\pm$ 0.4
<i>Geochelone denticulata</i> = <i>Chelonoidis denticulata</i> (Linnaeus, 1766)	1	3	907 $\pm$ 19	1.77 $\pm$ 0.32	230.0 $\pm$ 18.6
<i>Geochelone sulcata</i> (Miller, 1779)	2	44	255 $\pm$ 9	1.47 $\pm$ 0.09	114.0 $\pm$ 4.0
<i>Geochelone radiata</i> = <i>Astrochelys radiata</i> (Shaw, 1802)	1	53	248 $\pm$ 2	1.03 $\pm$ 0.02	115.8 $\pm$ 0.4
<i>Testudo kleinmanni</i> Lortet, 1883	1	98	464 $\pm$ 18	0.48 $\pm$ 0.02	516.3 $\pm$ 0.4
<i>Testudo h. hermanni</i> Gmelin, 1789	40	2196	144 $\pm$ 6	2.4 $\pm$ 0.1	602.1 $\pm$ 17.1
<i>Testudo h. boettgeri</i> Mojsisovics, 1889	2	48	209 $\pm$ 18	2.86 $\pm$ 0.12	535.3 $\pm$ 79.8
<i>Testudo graeca graeca</i> Linnaeus., 1758	13	789	462 $\pm$ 49	2.1 $\pm$ 0.2	391.3 $\pm$ 20.1
<i>Testudo marginata</i> Schoepff, 1793	19	1479	475 $\pm$ 4	1.6 $\pm$ 0.1	295.9 $\pm$ 1.0
<i>Indotestudo travancorica</i> (Boulenger, 1907)	1	2	483 $\pm$ 17	—	400.0 $\pm$ 20.0

with wavelengths approximately equal to or smaller than their body size (Bradbury & Vehrencamp, 1998).

Therefore, the aims of the present study are to review the courtship behaviour of chelonians in order (1) to explore the occurrence of mounting-calls and other vocalisations in this taxon by taking into account the evolutionary history and breeding habitat; and (2) to analyse the effects of body size on call frequency in a sample of calling species.

If tortoise calls are produced by a specific vibrating structure and function to convey information about the signaller to conspecifics, we may expect that (1) mounting-calls should mainly occur in species where an acoustic communication system based on air flow is more likely to be developed and may confer some advantages to signallers, i.e. in terrestrial or semi-terrestrial species; aquatic species of chelonians mate underwater (Ernst & Barbour, 1989; Kaufmann, 1992) and respiratory exchanges needed to produce sounds are unlikely; (2) the mounting-call should have a complex structure, showing harmonics and both frequency and amplitude modulation; these acoustic features are unique to vibrating systems, and are not present in sounds produced by simple air flow through larynx (Bradbury & Vehrencamp, 1998). For example, the hiss emitted by rattlesnakes (*Crotalus adamanteus*), which is a consequence of body inflation being produced only during the periods of rapid inhalation, is a

simple sound without a distinct amplitude and frequency modulation and harmonics (Kinney *et al.* 1998); (3) given the size-frequency relationship peculiar of sounds produced by vibrating structures (Ryan & Brenovitz, 1985; Ryan, 1985), call fundamental frequency should decrease with increasing body size among tortoises species; (4) call features should be related to some individual qualities increasing the signaller success in socio-sexual contexts. This last prediction was already verified in *Testudo marginata* (Sacchi *et al.* 2003) and *T. hermanni* (P. Galeotti & R. Sacchi, unpublished data), thus we focused on the first three predictions in this study.

## METHODS

Information about courtship behaviour of chelonians as a whole was collected from the most comprehensive review of the taxon (Ernst & Barbour, 1989). For each of the 246 recognised species we recorded courtship behaviour, habitat (marine, fresh-water, semi-terrestrial, and terrestrial) and the occurrence of mounting-calls. Since vocalisations are the most striking trait of tortoise's mating, we considered a species as not vocalising if calls were not explicitly reported in the courtship description. We also recorded the occurrence of vocalisation outside sexual contexts.

The acoustic structure of vocalisations was examined by recording 4776 mounting-calls of 83 males belong-

ing to 11 different species and sub-species of Testudinidae (Table 1) during spring-summer 2001-2002, at the European Centre for Tortoise Conservation ("CARAPAX", Massa Marittima, Tuscany, Central Italy), where 8000 individuals of several chelonian species reproduce in enclosures, in semi-natural conditions. We recorded the vocalisations using a Sony TCD-D7 DAT tape recorder connected to a SHURE shotgun microphone with a hypercardioid capsule. For each recorded species (or subspecies) we considered the carapace length as a measure of body size; we took the mean carapace length for species with many recorded individuals. *Testudo hermanni hermanni* Gmelin, 1789 and *T. hermanni boettgeri* Mojsisovics, 1889 were considered separately due to their difference in mean carapax size (14 and 19 cm respectively, Ernst & Barbour, 1989).

Recordings obtained from each male during courtship were analysed by the AVISOFT SAS-LAB pro software (©Specht, 1993). The best resolution was achieved analysing vocalisations in the 0-4 kHz frequency range, with a sampling rate of 8000 sample/s, frequency resolution 15 Hz and time resolution 8 ms. For each call we selected and measured directly on the screen the fundamental frequency in Hz; for species with many recorded individuals, we used the mean fundamental frequency.

#### PHYLOGENETIC ANALYSES AND STATISTICS

We produced two phylogenetic trees using the TreeEdit program (Rambaut & Charleston, 2001): the first included all chelonian genera encompassing vocal species and was used to trace vocalization appearance in the evolutionary history of chelonians (Fig. 1). This phylogeny was based on the molecular information by Shaffer *et al.* (1997), derived from cytochrome *b* and 12S ribosomal DNA sequences, combined with the phylogenetic trees on Chelidae Gray, 1825 from Seddon *et al.* (1997) and Georges *et al.* (1998, 2002); on Emydidae from Bickham *et al.* (1996), Lamb & Osentovsky (1997), Lindeman (2000) and Feldman & Parham (2002); and on Bataguridae from Parham *et al.* (2001). In the few cases where there was no completely bifurcating phylogeny available, we formed polytomies (nodes with more than two descendant taxa).

The second tree included only the 11 species and sub-species of Testudinidae we used to analyze the relationship between call fundamental frequency and body size, and was based on molecular information reported by Caccone *et al.* (1999a,b), Meylan (2000), and van der Kuyl *et al.* (2002). In this case, phylogeny was completely resolved (Fig. 2).

Data for different species cannot be considered as independent points in comparative studies because closely related species are more likely to share similar features due to a common ancestor (Felsenstein, 1985; Harvey & Pagel, 1991). However, there is debate about the importance of using comparative methods to control for shared evolutionary history (Ricklefs & Starck, 1996;

Price, 1997; Harvey & Rambaut, 1998), and it has been suggested that species-level analyses may, in some cases, be an appropriate method for analysis of comparative data (Ricklefs & Stark, 1996; Losos, 1999); in fact, differences between results using raw species data and phylogenetic methods may provide some biological insight (Price, 1997). Therefore, we analyzed our data using both the raw species values and comparative methods that control for phylogeny (statistically independent contrasts).

To produce data that were phylogenetically independent under a specific evolutionary model, we calculated standardized linear contrasts (Felsenstein, 1985; Harvey & Pagel, 1991) as implemented by the computer package Comparative Analysis of Independent Contrasts (CAIC, Purvis & Rambaut, 1995). The CAIC program produces linear contrasts that are standardized differences in traits at evolutionarily independent nodes in the phylogeny (Purvis & Rambaut, 1995). Since we have no information about branch lengths, contrasts were standardized assuming that lengths of branches in the phylogeny were either proportional to the number of taxa in each clade (Graphen, 1989), which is similar to a gradual model of evolution, or equal in length, which represents a punctuated model of evolution. In order to verify if branch lengths were appropriate, we computed Pearson correlation coefficients between the absolute value of each standardized independent contrast and its standard deviation (Garland *et al.* 1992). No significant relationships were found for both equal and proportional branch length (all  $r_p$  coefficients < 0.28), indicating that contrasts were adequately standardized. Our results were similar with graduated and punctuated branch lengths, so for simplicity we present just the results from the analysis using equal branch lengths.

For both raw species and independent contrasts analyses we used a linear regression model (LRM) to investigate whether variance in fundamental frequency among species was accounted for by body size. Fundamental frequency and carapace length values were  $\log_{10}$ -transformed. Regression for phylogenetic analysis was forced through the origin, because the mean value of independent contrasts is expected to be zero under the null hypothesis (Harvey & Pagel, 1991).

## RESULTS

#### OCCURRENCE AND EVOLUTION OF MOUNTING-CALLS

Courtship behaviour has been described for 101 chelonian species (Table 2). Among them, mounting-calls were reported for 35 species belonging to the families Testudinidae ( $n=29$ , 80.5% of species with described courtship), Trionychidae ( $n=3$ , 60% of species with described courtship), Emydidae ( $n=2$ , 7.4% of species with described courtship) and Bataguridae ( $n=1$ , 8.3% of species with described courtship). To our knowledge, none of the species belonging to other families has been reported to produce mounting-calls. However, we found

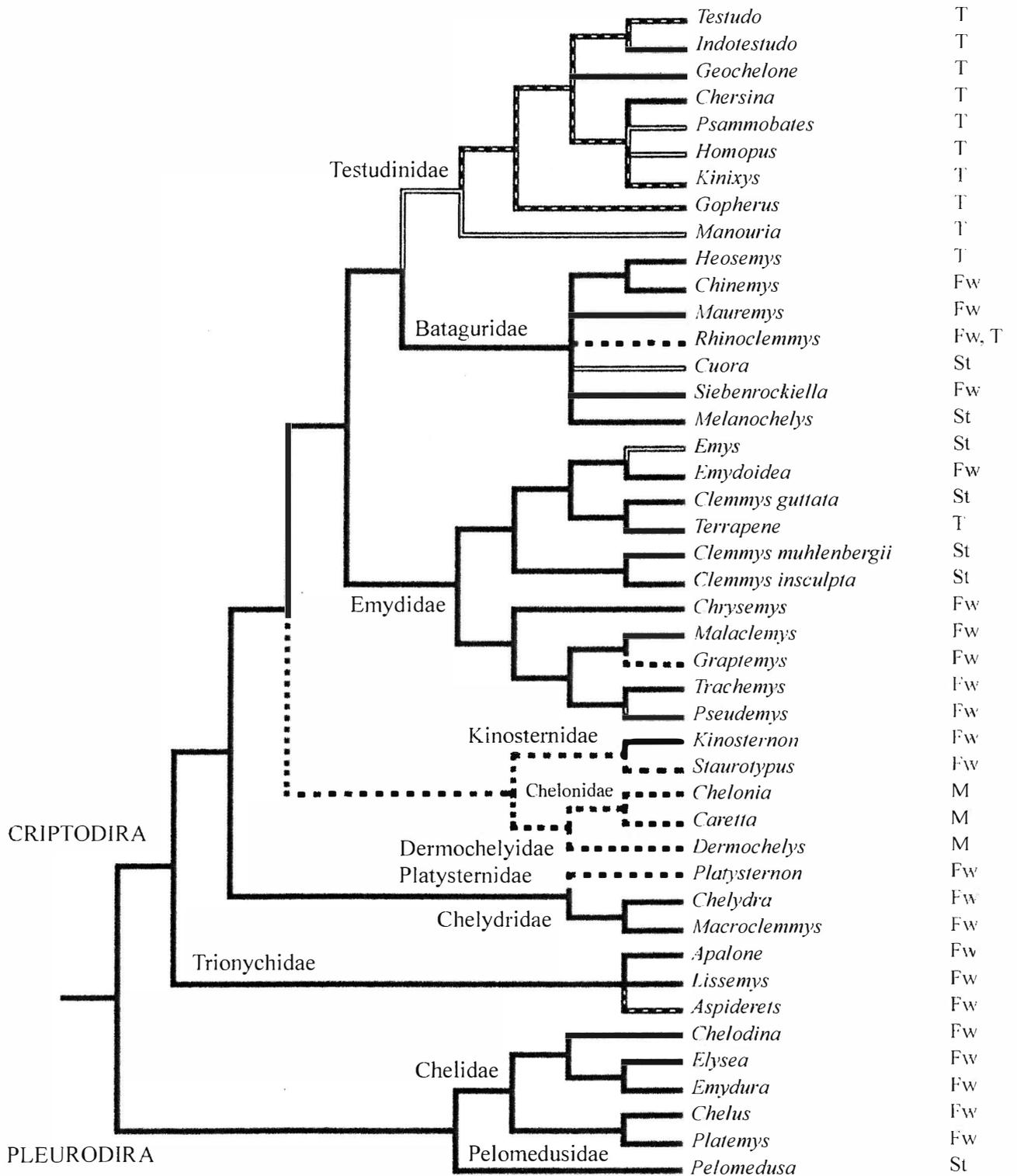


FIG. 1. Phylogenetic tree for chelonian genera (see Methods) whose courtship has been described. Black lines: genera that do not vocalize; white lines: genera including species vocalizing during mounting; black and white lines: genera including species vocalizing both during and outside mounting; dotted lines: genera including species vocalizing outside mounting. Single species are reported for polyphyletic genera. Main habitats are indicated on the right column: T, terrestrial; St, semi-terrestrial; Fw, fresh-water; M, marine.

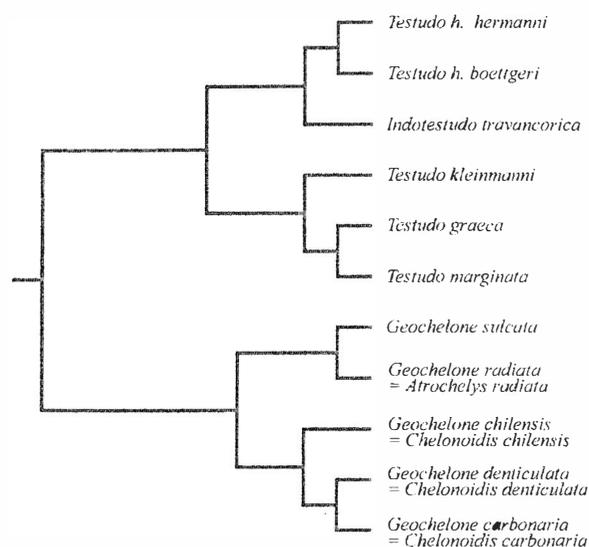


FIG. 2. Phylogenetic tree for the 11 species and sub-species of Testudinidae (see Methods) used to study relationship between body size and call fundamental frequency.

that other call types are used by some species of Cheloniidae Oppel, 1811, Dermochelyidae Fitzinger, 1843, Platysternidae Gray, 1869, Emydidae and Bataguridae outside courtship and mounting, for example during combats or when disturbed (Table 3); these kinds of vocalizations are emitted by both sexes in sev-

eral species, and also by juveniles of *Geochelone carbonaria* = *Chelonoidis carbonaria* (Spix, 1824) during foraging. Considering that courtship behaviour has been described, at least roughly, for only 41% of species, the ability to produce vocalisations should probably be more widespread in chelonians, and it is definitely not limited to sexual contexts.

The phylogenetic tree of chelonian genera we constructed (Fig. 1) indicated that calls associated with mounting were apparently absent in all Pleurodiran genera, appeared firstly in the ancestral Cryptodiran group of Trionychidae, were maintained in some Emydidae and Bataguridae genera, but were present in all recent Testudinidae, where mounting-calls appeared from the root of the family evolutionary tree (genus *Manouria* Gray, 1852), and was then inherited by the other genera. Therefore, the lack of mounting-calls in the genus *Chersina* Gray, 1831 may be considered a secondary loss.

It is worth noting that, apart from the three fresh-water species of Trionychidae, all the other vocal species are terrestrial or semi-terrestrial, while none of the marine species had been reported to vocalise during courtship and mounting. In fact most marine and fresh-water species mate underwater. Therefore, mounting-calls were significantly related to terrestrial habitats ( $\chi^2_1=33.7$ ,  $P<0.001$ ).

TABLE 2. Number and occurrence of chelonian species vocalizing during mount.

Family	Habitat	No. species	No. species with described courtship	% species that vocalize among those with described courtship
Chelidae Gray, 1825	fresh water	36	8	
Pelomedusidae Cope, 1868	fresh water	22	0	
	semi-terrestrial	1	1	
Carettochelyidae Boulenger, 1887	fresh water	1	0	
Trionychidae Fitzinger, 1826	fresh water	21	5	60.0
	unknown	1	0	
Chelydridae Gray, 1831	fresh water	2	2	
Platysternidae Gray, 1869	fresh water	1	0	
Cheloniidae Oppel, 1811	marine	6	2	
Dermochelyidae Fitzinger, 1843	marine	1	0	
Kinosternidae Agassiz, 1857	fresh water	22	8	
Dermatemydidae Gray, 1870	fresh water	1	0	
Emydidae Rafinesque, 1815	fresh water	28	21	4.8
	semi-terrestrial	4	3	33.3
	terrestrial	3	3	
Bataguridae = Geoemydidae Theobald, 1868	fresh water	37	7	
	semi-terrestrial	6	2	50.0
	terrestrial	7	2	
	unknown	6	0	
Testudinidae Batsch, 1788	terrestrial	40	35	80.5
Total		246	101	34.6

TABLE 3. Description, emitting sex and context of vocalizations uttered by turtles and tortoises (M: male, F: female, J: juvenile).

Species	Vocalization	Sex	Context	References
<i>Dermochelys coriacea</i> (Vandelli, 1761)	wail/groans/roar/bellow	M	when attacked	Carr 1952
	roars	M	diving	Kumpf 1964
	?	F	nesting	Mrosofsky 1972
"Sea turtles"	"mercy cry"/roar/grunt	?	?	Carr 1952
<i>Aspideretes hurum</i> (Gray, 1831)	?	M	courtship	Flower 1899
<i>Aspideretes nigricans</i> (Anderson, 1875)	hiss	?	?	Annandale (in Ernst & Barbour 1989)
<i>Aspideretes gangeticus</i> (Cuvier, 1824)	low, hoarse cackle	?	courtship?	Günther 1864
<i>Staurotypus triporcatus</i> (Wiegmann, 1828)	2 voices	?	?	Cope 1865
<i>Platysternon megacephalum</i> Gray, 1831	squeal	J	when disturbed	Campbell & Evans 1972
<i>Rhinoclemmys punctularia</i> (Daudin, 1801)	piping	?	when killed	Cope 1865
<i>Cuora flavomarginata</i> = <i>Cistoclemmys flavomarginata</i> Gray, 1863	lip-smacking/hiss	M	courtship	Connor & Wheeler 1998
<i>Emys orbicularis</i> (Linnaeus, 1758)	low piping note	M	mounting	M. Zuffi, M. Lebboroni, pers. com.
<i>Pseudemys floridiana</i> (Le Conte, 1830)	grunt	?	courtship?	Neil 1950
<i>Graptemys</i> Agassiz, 1857 sp.	?	?	?	cited in Campbell & Evans 1972
<i>Kinixys belliana</i> Gray, 1831	wheezing honks	M/F	combat/ mounting	Morris 1994
<i>Kinixys erosa</i> (Schweigger, 1812)	hissing squeak	M	mounting	Ernst & Barbour 1989
<i>Kinixys homeana</i> Bell, 1827	moaning hiss	M	mounting	Kirkpatrick 1998
<i>Kinixys natalensis</i> Hewitt, 1935	moaning hiss	M	mounting	Kirkpatrick 1998
<i>Homopus signatus</i> (Gmelin, 1789)	squeaking/loud squeak	M	courtship/ mounting	Palmer 1994
<i>Psammobates oculifera</i> = <i>Psammobates oculiferus</i> (Kuhl, 1820)	grunting cough	M	mounting	Ernst & Barbour 1989
<i>Manouria emys</i> (Schlegel & Müller, 1840)	moan	M/F	courtship/ mounting	McKeown <i>et al.</i> 1990
<i>Indotestudo elongata</i> (Blyth, 1853)	harsh raspy sound	M/F	combat/ mounting	McCormick 1992, C. Tabaka, pers. com.
<i>Indotestudo forstenii</i> (Schlegel & Müller, 1840)	?	M/F	combat/ mounting	C. Tabaka, pers. com.
<i>Indotestudo travancorica</i> (Boulenger, 1907)	pulses	M	nocturnal/ chorus	Auffenberg (in Campbell & Evans 1972)
	grunt	M	mounting	Auffenberg 1964
<i>Geochelone elegans</i> (Schoepff, 1794)	grunt	M	mounting	Ernst & Barbour 1989
<i>Geochelone platynota</i> (Blyth, 1863)	grunt-like bellow	M	mounting	<a href="http://www.startortoise.com">www.startortoise.com</a>
<i>Geochelone pardalis</i> (Bell, 1828)	grunt-like bellow	M	mounting	Ernst & Barbour 1989
<i>Geochelone sulcata</i> (Miller, 1779)	grunt or ducklike quack	M	mounting	Grubb 1971

continued...

TABLE 3. continued...

<i>Geochelone nigra</i> = <i>Chelonoidis nigra</i> (Quoy & Gaimard, 1824)	roar/bellow	M	mounting	De Sola 1930, Evans 1949, Van Denburgh 1914
<i>Geochelone gigantea</i> = <i>Dipsochelys elephantina</i> (Duméril & Bibron, 1835)	groan/bellow	M	mounting	Ernst & Barbour 1989
<i>Geochelone radiata</i> = <i>Astrochelys radiata</i> (Shaw, 1802)	grunt	M	mounting	Ernst & Barbour 1989
<i>Geochelone yniphora</i> = <i>Astrochelys yniphora</i> (Vaillant, 1885)	?	M	mounting	Ernst & Barbour 1989
<i>Geochelone carbonaria</i> = <i>Chelonoidis carbonaria</i> (Spix, 1824)	cluck cluck or chirp	M J	mounting, foraging	Auffenberg 1965, Campbell & Evans 1967, Campbell 1967
<i>Geochelone denticulate</i> = <i>Chelonoidis denticulata</i> (Linnaeus, 1766)	cluck	M	mounting	Snedigar & Rokosky 1950, Auffenberg 1965
<i>Geochelone chilensis</i> = <i>Chelonoidis chilensis</i> (Gray, 1870)	grunt or ducklike quack	M	mounting	present paper
<i>Testudo graeca</i> Linnaeus, 1758	whistlelike noise	M/F	combat/ mounting	Ernst & Barbour 1989 present paper
<i>Testudo hermanni</i> Gmelin, 1789	squeaklike grunt	M/F	combat/ mounting	Ernst & Barbour 1989 present paper
<i>Testudo horsfieldii</i> = <i>Agriemys horsfieldii</i> (Gray, 1844)	cluck/squeak	M	mounting	Cohen 1994
<i>Testudo kleinmanni</i> Lortet, 1883	interrupted rattling	M	mounting	Hoofien 1971
<i>Testudo marginata</i> Schoepff, 1793	guttural utterance	M/F	combat/ mounting	Hine 1982, present paper
<i>Gopherus agassizii</i> (Cooper, 1863)	grunt/moan	M	exploration/ mounting	Ernst & Barbour 1989, Campbell & Evans 1967
<i>Gopherus berlandieri</i> (Agassiz, 1857)	?	M	mounting	Hoseholder (in Weaver 1970)
<i>Gopherus polyphemus</i> (Daudin, 1801)	rasping all/mew/ piteous cry	M	?	Carr 1952, Hallinan 1923

## CALL FEATURES AND RELATIONSHIP WITH BODY SIZE

In the species we recorded, tortoises emitted a long sequence of simple calls with regular intervals between them during mount (see Table 1 for details). In both *Testudo* Linnaeus, 1758 (Fig. 3) and *Indotestudo* Lindholm, 1929 genera, the calls sounded like whimpers or bellows, and were highly stereotyped within each individual. Calls were highly modulated in frequency and showed a clear harmonic structure: in *T. marginata* up to 13 harmonics could be observed. The call fundamental frequency in these two genera ranged from 296 Hz of *T. marginata* to 602 Hz of *T. h. hermanni*.

In the genus *Geochelone* Fitzinger, 1835 and *Chelonoidis* Fitzinger, 1835 vocalisations consisted in a series of short "clucks" repeated at regular intervals (Fig. 3). Fundamental frequency in this genus ranged from 110 Hz of *C. carbonaria* Fitzinger, 1835 to 230 Hz

of *G. denticulate* = *Chelonoidis denticulata* (Linnaeus, 1766).

The LRM using raw species data showed that call fundamental frequency was negatively related to carapace length ( $F_{1,10}=17.77$ ,  $P=0.002$ ,  $\beta=-0.81$ ,  $R^2=0.66$ ); after controlling for phylogeny, the negative relationship between fundamental frequency and carapace length still persisted ( $F_{1,9}=6.33$ ,  $P=0.033$ ,  $\beta=-0.44$ ,  $R^2=0.41$ ). Thus, larger tortoise species emitted calls at lower frequencies than smaller ones (Fig. 4).

## DISCUSSION

Mounting-calls occur in 35% of chelonian species whose courtship behaviour has been described. This figure probably underestimates the real occurrence of mounting calls, due to our limited knowledge of chelonian mating behaviour. Nevertheless, it indicates that

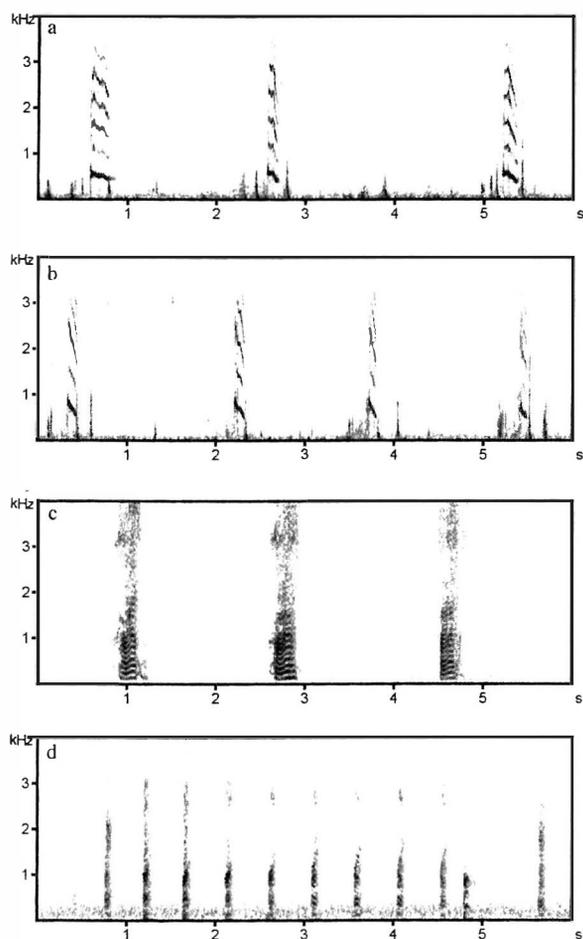


FIG. 3. Sonograms of mounting-calls emitted by (a) *Testudo hermanni boettgeri*, (b) *Testudo hermanni hermanni*, (c) *Geochelone sulcata* and (d) *Geochelone = Chelonoidis carbonaria*. (FFT: 512, frame: 100%, overlap: 87.5%, window: Flap Top, bandwidth: 59 Hz).

the ability to produce sounds is more widespread in this taxon than previously thought. Moreover, vocalisations are also used outside sexual contexts. The phylogenetic tree we constructed for chelonian genera revealed that mount vocalisations probably evolved in a cryptodirian ancestor, were maintained in some subsequent genera and lost in others, but spread out in all terrestrial Testudinidae. In fact, only four families include species vocalising during mount and most of them (82.8%) belong to Testudinidae. Since vocalizations are maintained throughout the evolutionary history of chelonians, it is likely that mounting calls are functional, considering also that they may attract predators and are costly to produce. For example, in *T. hermanni* both singing rate and call frequency range of mounting males correlate positively with the haematocrit value, and call duration honestly reflects lymphocyte concentration in the peripheral blood (P. Galeotti & R. Sacchi, unpublished data), suggesting that calling requires both a high aerobic capacity (Chappell *et al.* 1997) and good general health condition. The fact that most Testudinidae species vocalise during mating therefore suggests that mounting-calls provide receivers with some useful in-

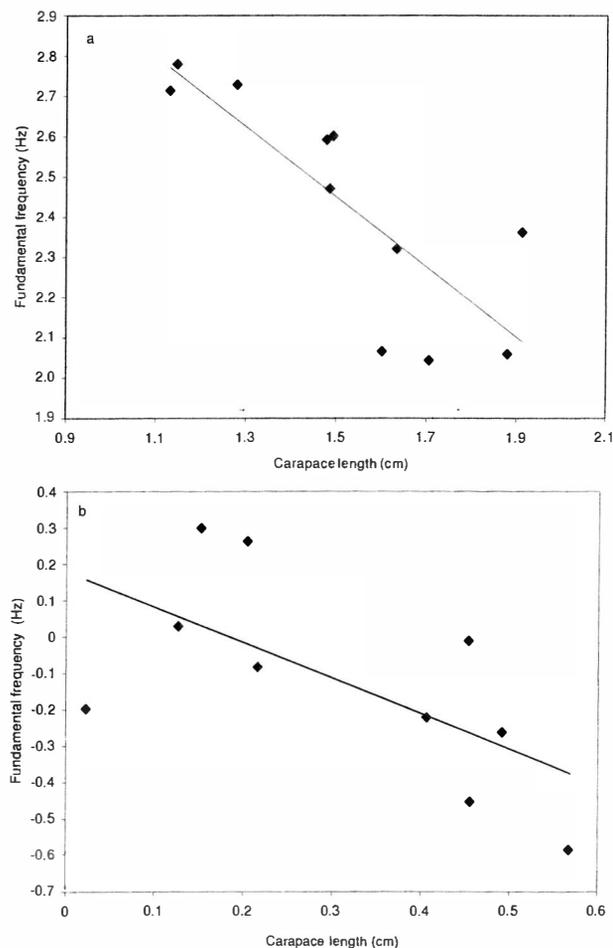


FIG. 4. Relationship between the call fundamental frequency (Hz) and carapace size (cm) for 11 species and subspecies of Testudinidae (a: raw species, b: independent contrasts).

formation to assess signaller qualities. Signallers, in turn, may gain some selective advantages, ranging from being preferred as sexual partners by females, as documented by their mounting success (Sacchi *et al.*, 2003; Galeotti *et al.*, 2005), to avoiding sexual interferences from other males.

The first line of evidence that mounting-calls function in intraspecific communication comes just from the fact that most chelonian species vocalising during mount are terrestrial or semi-terrestrial, and only three species live in fresh-waters, which is consistent with the lower costs of sound production in terrestrial habitats compared to aquatic ones (an aquatic sender will have to generate sounds with pressure levels 5000 times higher than in air just to provide the same energetic stimulation at receiver's ears, Bradbury & Vehrencamp, 1998). This finding strongly suggests that vocalisations evolved for communicating in terrestrial habitats. If vocalisations were only a by-product of copulatory efforts, they would occur in both terrestrial and aquatic species. However, to the best of our knowledge, no aquatic species produce mounting-call, even in those species where males emerge from water during mount.

The second line of evidence is that vocalisations of tortoise species we recorded showed both frequency and amplitude modulation, and a rich harmonic structure, which are acoustic features commonly used by birds and mammals (Catchpole & Slater, 1995; Manser, 2001) to encode information in intraspecific communication, and are incompatible with mechanisms of sound production based simply on air flow through respiratory tracts; they rather imply the presence of vibrating structures. For example, in a recent paper on the colubrid *Pituophis melanoleucus*, which emits hisses showing a clear harmonic structure, Young *et al.* (1995) found a specific acoustic device that he described as a 'vocal cord'. The evolution of such a complex structure seems to rule out a non-functional explanation for mounting-calls, at least in tortoises.

A third line of evidence that tortoise calls were not simple respiratory noises was supplied by the relationship between call fundamental frequency and carapace length. In both amphibians and birds, this relationship is largely due to the correlation of body size with the mass of the vibrating device producing sounds (Bowman, 1983; Ryan, 1985; Ryan & Brenovitz, 1985). The existence in tortoise species we recorded of the same size-dependent pattern of frequency further supports the existence of specific sound producing structures very similar to those found in other vertebrates.

The analyses we performed on the larynx morphology of three species of Testudinidae demonstrated the existence of fine anatomical structures not yet described: two blind *diverticula* on the bottom of the cricoid structures in *T. graeca* Linnaeus, 1758 and *T. marginata*. Moreover we found two belts of elastic fibres in the larynx wall, that may vibrate under air flow pressure thus generating sounds (Sacchi *et al.*, 2004).

A final consideration supporting the communicative function of mounting vocalisations in this taxon may be added: in all chelonian species for which a call spectrographic analysis has been carried out, the call fundamental frequency generally fell just within the range of ear sensitivity of these species (Wever & Vernon, 1956a,b; Gulick & Zwick, 1966; Patterson, 1966; Campbell & Evans, 1967; Ridgway *et al.* 1969).

On the whole, these findings appear to rule out the possibility that vocalisations in tortoises are simple "noises", involuntarily produced by copulatory movements (Weaver, 1970; Mrosovsky, 1972). Based on our study of *T. marginata* (Sacchi *et al.*, 2003) and *T. hermanni* (Galeotti *et al.*, 2005) we suggest that mount vocalisations are reliable condition-dependent signals conveying honest information about male body size, general health condition and mating success to conspecifics.

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#### REFERENCES

- Auffenberg, W. (1964). Notes on the courtship of the land tortoise *Geochelone travancorica* (Boulenger). *Journal of the Bombay Natural History Society* **61**, 247-253.
- Auffenberg, W. (1965). Sex and species discrimination in two sympatric South American tortoises. *Copeia* **1965**, 335-342.
- Berry, J. F. & Shine R. (1980). Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia* **44**, 185-191.
- Bickham, J. W., Lamb, T., Minx, P. & Patton J. C. (1996). Molecular systematic of the genus *Clemmys* and the intergeneric relationships of emydid turtles. *Herpetologica* **52**, 89-97.
- Bowman, R. I. (1983). The evolution of song in Darwin's finches. In *Patterns of evolution in Galapagos organisms*, 237-537. Bowman, R. I., Berson, M. and Leviton, A. E. (Eds.). San Francisco: American Association for the Advancement of Science.
- Bradbury, J. W. & Vehrencamp, S. L. (1998). *Principles of animal communication*. Sunderland, Massachusetts: Sinauer Associates.
- Caccone, A., Amato, G., Gratry, O. C., Behler, J. & Powell, J. R. (1999a). A molecular phylogeny of four endangered Madagascar tortoises based on MtDNA sequences. *Molecular Phylogenetics and Evolution* **12**, 1-9.
- Caccone, A., Gibbs, J. P., Ketmaier, V., Suatoni, E. & Powell, J. R. (1999b). Origin and evolutionary relationships of giant Galapagos tortoises. *Proceedings of the National Academy of Sciences of the USA* **9**, 13223-13228.
- Campbell, H. W. (1967). Stop, look, and listen. Acoustic behaviour of turtles. *Journal of the International Turtle and Tortoise Society* **1**, 13-44.
- Campbell, H. W. & Evans, W. E. (1967). Sound production in two species of tortoises. *Herpetologica* **23**, 204-209.
- Campbell, H. W. & Evans, W. E. (1972). Observation of the vocal behavior of chelonians. *Herpetologica* **28**, 277-280.
- Carr, A. F. J. (1952). *Handbook of Turtles*. Ithaca, New York: Comstok.
- Catchpole, C. K. & Slater, P. J. B. (1995) *Bird song, biological themes and variations*. Cambridge: Cambridge University Press.
- Chappell, M. A., Zuk, M., Johnsen, T. S. & Kwan, T. H. (1997). Mate choice and aerobic capacity in red junglefowl. *Behaviour* **134**, 511-529.
- Cohen, M. A. (1994). Russian Tortoise, *Testudo horsfieldii*. *Tortuga Gazette* **30**, 1-4.
- Connor, M. J. & Wheeler, V. (1998). The Chinese box turtle, *Cistoclemmys flavomarginata* Gray 1863. *Tortuga Gazette* **34**, 1-7.

- Cope, E. D. (1865). Third contribution to the herpetology of tropical America. *Proceedings of the National Academy of Sciences of the USA* **173**, 185-196.
- De Sola, C. R. (1930). The Liebesspiel of *Testudo vandenberghii*, and a new name for the mid-Albemarle Island Galapagos Tortoise. *Copeia* **1930**, 79-80.
- Ernst, C. H. & Barbour, R. W. (1989). *Turtles of the World*. Washington: Smithsonian Institution Press.
- Evans, W. E. (1949). The reproductive behaviour of the giant tortoises *T. vicina* and *T. vandenberghii*. *Anatomical Record* **105**, 579.
- Feldman, C. R. & Parham, J. F. (2002). Molecular phylogenetics of Emydidae Turtles: taxonomic revision and the evolution of shell kinesis. *Molecular Phylogenetics and Evolution* **22**, 388-398.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist* **125**, 1-15.
- Flower, S. S. (1899). Notes on a second collection of reptiles made in the Malay Peninsula and Siam, from November 1896 to September 1898, with a list of species recorded from those countries. *Proceedings of the Zoological Society (London)* **1899**, 600-696.
- Galeotti, P., Sacchi, R., Pellitteri Rosa, D. & Fasola, M. (2005). Female preferences for fast-rate, high-pitched calls in Hermann's tortoises *Testudo hermanni*. *Behavioral Ecology* **16**, 301-308.
- Gans, C. & Maderson, P. F. A. (1973). Sound production mechanisms in recent reptiles: review and comment. *The American Zoologist* **13**, 1195-1203.
- Garland, T., Harvey, P. H. & Ives, A. R. (1992). Procedures for the analyses of comparative data using phylogenetically independent contrasts. *Systematic Biology* **41**, 18-32.
- Georges, A., Birrel, J., Saint, K. M., McCord, W. & Donnellan, S. C. (1998). A phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nucleolar gene sequence variation. *Biological Journal of the Linnean Society* **67**, 213-246.
- Georges, A., Adams, M. & McCord, W. (2002). Electrophoretic delineation of species boundaries within the genus *Chelodina* (Testudines: Chelidae) of Australia, New Guinea and Indonesia. *Zoological Journal of the Linnean Society* **134**, 401-421.
- Graphen, A. (1989). The phylogenetic regression. *Philosophical Transactions of the Royal Society* **326**, 119-156.
- Grubb, P. (1971). Comparative notes on the behaviour of *Geochelone sulcata*. *Herpetologica* **27**, 328-332.
- Gulick, W. L. & Zwick, H. (1966). Auditory sensitivity of the turtle. *Psychological Research* **16**, 47.
- Günther, A. (1864). *Reptiles of British India*. London: Robert Hardwicks.
- Hallinan, T. (1923). Observations made in Duval Co., Northern Florida, on the gopher tortoise (*Gopherus poliphemus*). *Copeia* **1923**, 11-20.
- Harvey, P. H. & Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- Harvey, P. H. & Rambaut, A. (1998). Phylogenetic extinction rates and comparative methodology. *Proceedings of the Royal Society B: Biological Sciences* **265**, 1691-1696.
- Hine, M. (1982). Notes on the marginated tortoise (*Testudo marginata*) in Greece and captivity. *The Bulletin of the British Herpetological Society* **5**, 35-38.
- Hoofien, J. H. (1971). The voices of snakes and tortoises. *Israel Journal of Zoology* **20**, 148.
- Kaufmann, J. H. (1992). The social behaviour of Wood Turtles, *Clemmys insculpta*, in Central Pennsylvania. *Herpetological Monographs* **6**, 1-25.
- Kinney, C., Abishahin, G. & Young, B. A. (1998). Hissing in rattlesnakes: redundant signalling or inflationary epiphenomenon? *Journal of Experimental Zoology* **280**, 107-113.
- Kirkpatrick, D. T. (1998). African hingeback tortoises of the genus *Kinixys*. *Reptile & Amphibian Magazine* **54**, 32-37.
- Kumpf, H. E. (1964). Use of underwater television in bio-acoustic research. In *Marine bio-acoustic*, 45-57. Tavalga, W. N. (Ed.). Oxford: Pergamon Press.
- Lamb, T., & Osentovski, M. F. (1997). On the parafyly of *Malaclemys*: a molecular genetic assessment. *Journal of Herpetology* **31**, 258-265.
- Lindeman, P. V. (2000). Resources use of five sympatric turtle species: effects of competition, phylogeny, and morphology. *Canadian Journal of Zoology* **78**, 992-1008.
- Losos, J. B. (1999). Uncertainty in the reconstruction of ancestral character states and limitations on the use of phylogenetic comparative methods. *Animal Behaviour* **58**, 1319-1324.
- Manser, M. B. (2001). The acoustic structure of suricate's alarm calls varies with predator type and the level of response urgency. *Proceedings of the Royal Society B: Biological Sciences* **268**, 2315-2324.
- McCormick, B. (1992). The elongated tortoise, *Indotestudo elongata*. *Tortuga Gazette* **28**, 1-3.
- McKeown, S., Meier, D. E. & Juvik, J. O. (1990). The management and breeding of the Asian forest tortoise (*Manouria emys*) in captivity. In *Proceedings of the first International Symposium on Turtles and Tortoises: conservation and captive husbandry*, 138-159. Beaman, K. R., Caporaso, F., McKeown, S. & Graff, M. (Eds.). Van Nuys: California Turtle and Tortoise Club.
- Meylan, P. A. (2000). *Hesperotestudo* (Testudines: Testudinidae) from the Pleistocene of Bermuda, with comments on the phylogenetic position of the genus. *Zoological Journal of the Linnean Society* **128**, 51-76.
- Morris, L. (1994). Western hinge-back tortoise, *Kinixys belliana nogueyi*. *Tortuga Gazette* **30**, 1-3.
- Mrosovsky, N. (1972). Spectrographs of the sounds of leatherback turtles. *Herpetologica* **28**, 256-258.
- Neil, W. T. (1950). Sound produced by the Suwanee terrapin. *Copeia* **1950**, 62.
- Olsson, M., & Madsen, T. (1998). Sexual selection and sperm competition in reptiles. In *Sperm competition*

- and sexual selection, 503-564. Birkhaed, T. R. & Møller, A. P. (Eds.). Stockholm: Morgan Kaufmann.
- Palmer, M. 1994. The speckled tortoise, *Homopus signatus*, in captivity. *Tortuga Gazette* **30**, 1-5.
- Parham, J. F., Simison, W. B., Kozak, K. H., Feldman, C. R. & Shi, H. (2001). New Chinese turtles: endangered or invalid? A reassessment of two species using mitochondrial DNA, allozyme electrophoresis and known-locality specimens. *Animal Conservation* **4**, 357-367.
- Patterson, W. C. (1966). Hearing in the turtle. *Journal of Auditory Research* **6**, 453-464.
- Pope, C. H. (1955). *The Reptile World*. New York: Knopf.
- Price, T. (1997). Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society* **352**, 519-529.
- Purvis, A. & Rambaut, A. (1995). Comparative analysis by independent contrasts CAIC: an Apple Macintosh application for analysing comparative data. *Computer Applications in the Biosciences* **11**, 247-251.
- Rambaut, A. & Charleston, M. (2001). *TreeEdit: phylogenetic tree editor v. 1.0 alpha 8*. University of Oxford.
- Ricklefs, R. E. & Starck, J. M. (1996). Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* **77**, 167-172.
- Ridgway, S. H., Wever, E. G., McCormick, J. G., Palin, J. & Anderson, J. H. (1969). Hearing in the giant sea turtle, *Chelonia mydas*. *Proceedings of the National Academy of Sciences of the USA* **64**, 884-890.
- Ryan, M. J. (1985). *The Tungara frog*. Chicago: University of Chicago Press.
- Ryan, M. J. & Brenowitz, E. A. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *The American Naturalist* **126**, 87-100.
- Sacchi, R., Galeotti, P., Fasola, M. & Ballasina, D. (2003). Vocalizations and courtship intensity correlate with mounting success in marginated tortoises *Testudo marginata*. *Behavioural Ecology and Sociobiology* **55**, 95-102.
- Sacchi, R., Galeotti, P., Fasola, M. & Gerzeli, G. (2004). Larynx morphology and sound production in three species of Testudinidae. *Journal of Morphology* **26**, 175-183.
- Seddon, J. M., Georges, A., Baverstock, P. R. & McCord, W. (1997). Phylogenetic relationships of chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA gene sequence variation. *Molecular Phylogenetics and Evolution* **7**, 55-61.
- Shaffer, H. B., Meylan, P. & McKnight, M. L. (1997). Tests of turtle phylogeny: molecular, morphological, and paleontological approaches. *Systematic Biology* **46**, 235-268.
- Snedigar, R. & Rokosky, E. J. (1950). Courtship and egg laying of captive *Testudo denticulata*. *Copeia* **1950**, 46-48.
- Swingland, I. & Stubbs, D. (1985). The ecology of a Mediterranean tortoise (*Testudo hermanni*): reproduction. *Journal of Zoology (London)* **205**, 595-610.
- Van Denburgh, J. (1914). The giant land tortoises of the Galapagos Arcipelago. *Proceedings of the Californian Academy of Sciences* **2**, 203-374.
- Van Der Kuyl, A. C., Ballasina, D. L. P., Dekker, J. T., Maas, J., Willemsen, R. E. & Goudsmit, J. (2002). Phylogenetic relationships among the species of the genus *Testudo* (Testudines: Testudinidae) inferred from mitochondrial 12S rRNA gene sequences. *Molecular Phylogenetics and Evolution* **22**, 174-183.
- Weaver, W. G. Jr. (1970). Courtship and combat behaviour in *Gopherus berlandieri*. *Bulletin of the Florida State Museum* **15**, 1-43.
- Wever, E. G. & Vernon, J. A. (1956a). The sensitivity of the turtle's ear as shown by its electrical potentials. *Proceedings of the National Academy of Sciences of the USA* **42**, 213-220.
- Wever, E. G. & Vernon, J. A. (1956b). Sound transmission in the turtle's ear. *Proceedings of the National Academy of Sciences of the USA* **42**, 292-299.
- Young, B. A., Sheft, S. & Yost, W. (1995). Sound production in *Pituophis melanoleucus* (Serpentes: Colubridae) with the first description of vocal cord in snakes. *Journal of Experimental Biology* **273**, 472-481.

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## EFFECTIVENESS OF AMPHIBIAN MONITORING TECHNIQUES IN A TAIWANESE SUBTROPICAL FOREST

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We compared the effectiveness of three techniques for surveying frogs in four different habitats in a subtropical area of Taiwan. We conducted surveys biweekly from July 2000 to July 2001 employing three sampling techniques (nocturnal line-transects, automated recording systems, and side-flap pail-traps) concurrently in each of four habitats (temporary pond, permanent pond, ephemeral stream, and permanent marsh). We detected 22 species of anurans from five families, representing 76% of the anuran species found in Taiwan. Line-transect sampling and automated recorders detected 22 and 20 species respectively, with an average of  $12.3 \pm 3.2$  (mean  $\pm$  SD) and  $10.4 \pm 3.5$  species per survey. In contrast, traps only captured 11 species, with an average of  $2.1 \pm 1.5$  species per survey. Automated recorders were most effective at detecting hylids, rhacophorids, and microhylids that have loud calls and/or prolonged periods of calling. Recorders were less effective at detecting ranid and bufonid species that have relatively quiet calls. Traps were good at capturing most of the ranids, species that were usually missed with automated recorders. The combination of recorders and traps was equivalent to line-transect sampling, suggesting that these two techniques are a good alternative to nocturnal line-transect sampling, a technique that is difficult to use in remote areas and/or habitats that are inaccessible at night.

*Key words:* amphibian, sampling, survey methods, transect, trapping

### INTRODUCTION

Recent concerns about amphibian declines (Barinaga, 1990; Fellers & Drost, 1993; Drost & Fellers, 1996; Berger *et al.*, 1998; Lips, 1999; Davidson *et al.*, 2001, 2002) have encouraged the development and standardization of surveying and monitoring methods for amphibians (Heyer *et al.*, 1994). The standardization of sampling techniques allows researchers and conservationists worldwide to implement effective and accurate surveying programs. Sampling techniques vary with respect to cost, time investment, personnel requirements, and effectiveness. Thus, it is critical for researchers to test and implement methods that are most appropriate for their regions (Fellers & Freel, 1995; Parris, 1999; Corn *et al.*, 2000). Numerous studies have compared the effectiveness and suitability of amphibian sampling techniques. Some of this work has suggested that nocturnal line-transect sampling is particularly effective (Pearman *et al.*, 1995; Parris, 1999; Parris *et al.*, 1999). One major drawback is that line transects cannot be used in areas that are inaccessible to researchers at night, a situation that is often the case in tropical and subtropical forests. Therefore, it is important to evaluate alternative sampling techniques.

Automated recording systems have been used for a number of years, but the technique has been evaluated only in a limited variety of habitats. An automated re-

order has the advantage of allowing researchers to collect data for an extended period without disturbance to the study animals, and it can be used in areas that are difficult to access at night. Parris *et al.* (1999) compared automated recorders with other sampling techniques and found that recorders detected 71% of the species in a study in southeast Queensland, Australia, while line-transect sampling accounted for 93% of the species.

Our study was designed to compare three amphibian-sampling techniques in a subtropical forest in central Taiwan. We used nocturnal line-transect sampling, automated tape recorders, and side-flap pail-traps. Side-flap pitfall traps are good for sampling ground-dwelling frogs, but are ineffective for some species, especially arboreal frogs such as hylids (Nadorozny & Barr, 1997). One advantage of the trap is that it can be used in areas where it can be deployed and maintained during the day. Our work was designed to determine if the combination of automated recorders and traps was as effective at detecting anurans as nocturnal line transects across a variety of habitats. Most research on sampling techniques has been conducted in North and South America, and in Australia (Heyer *et al.*, 1994; Parris *et al.*, 1999; Bridges & Dorcas, 2000; Corn *et al.*, 2000); much less attention has been given to other areas, especially tropical and subtropical regions. The anuran community, as well as other biotic components, and the physical environment vary among regions. Thus, it is necessary to evaluate the effectiveness of sampling techniques in distinctly different settings.

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## MATERIALS AND METHODS

### STUDY AREA

Field work was conducted from July 2000 to July 2001 at the Taiwan Forestry Research Institute experimental forest at Lien Hua Chih station (23°55' N, 120° 52'E), Nantou County, in central Taiwan. The station encompasses a 461-ha watershed, half of which is covered by undisturbed lowland primary forest. The site is characterized by low topography, with elevations ranging from 576 to 975 m. The mean annual air temperature is 21.1°C, and ranges from 9.9°C in January to 30.0°C in July. The area receives approximately 220 cm of rain annually. Although rain falls in all months, the wet season begins in May and ends in October. Heavy rains are most often associated with the "plum rains" or "Mei rains" in spring (April - June) and the typhoon season in summer (July - September). The "plum rain" season is characterized by continuous light rain for many days, with occasional heavy thunderstorms. Typhoons usually bring heavy rains that can cause flash floods and damage to forests.

### SURVEY SITES

We chose one study site in each of four different habitats: a temporary pond, permanent pond, ephemeral stream, and permanent marsh. The temporary pond was filled with water in the spring and summer (March to September), but dried up in the fall and winter (October to February). The pond was surrounded by elephant grass (*Pennisetum purpureum*) and bamboo stands (*Phyllostachys* sp.). It covered approximately 400 m<sup>2</sup> during the wet season. The permanent pond was a 450-m<sup>2</sup> abandoned paddy field that held water all year, even though the water level dropped during the dry season. The ephemeral stream was 1-3 m wide and 20-30 cm deep during the wet season, with water passing through riffles and pools. During the dry season, reduced flow resulted in the formation of intermittent pools. The stream bank and bottom consisted of sand, gravel, boulders, and bedrock. The stream was completely shaded by broadleaf trees: *Michelia formosana*, *Adenantha microsperma*, *Pellionia radicans*, and *Entada phaseoloides*. The shallow, permanent marsh was 25 m<sup>2</sup> in area and fed by a spring. The dominant vegetation was *Juncus effusus*, *Cynodon dactylon*, and *Dicranopteris linearis*. The marsh was located at the edge of a primary forest consisting of *Michelia formosana*, *Gordonia axillaries*, *Cyathea metteniana*, and *Prunus campanulata*.

### SAMPLING TECHNIQUES

We surveyed biweekly from July 2000 to July 2001, employing three techniques concurrently at each site: nocturnal line transects, automated recording systems, and side-flap pail-traps. In nocturnal line-transect sampling, two people with headlights walked slowly along a 100-m transect, spotlighting for frogs within 2 m of the transect line. All four transects were surveyed on the

same night, beginning at 1900 hr. Each transect took 30-45 min; all four were finished in 3-4 hr. The sequence of transects was randomly selected each night. We counted the number of frogs of each species that were seen or heard.

We used automated tape recorders to record frog calls over a 12-hr period that coincided with the trapping and line transects. The system consisted of a AIWA TP-VS480 tape recorder (with AE-120 TDK tapes), a 12 V rechargeable battery, a timer (CEC, Type-CTW) housed in a waterproof box, and an AIWA stereo external microphone secured to a tree approximately 2 m above the ground. Each recorder began recording at 1900 hr, recorded for 1 min, and then turned off for 11 min; this cycle was repeated until 0700 hr the next morning. In theory, we would obtain a total of 60 min of recording each night, but we only obtained about 57-58 min each night due to the delay of the timer. We put one recorder at each end of the line transect, and the results from these two stations were pooled for each night. Tape recordings were played back in the laboratory. We identified each vocalization to species.

We trapped amphibians using modified side-flap pail-traps modeled after Nadorozny & Barr (1997). The trap was 60 × 30 × 35 cm (length × width × depth). An 8 × 8 cm entrance opening was cut in the side of the box, 8 cm up from the bottom, and a 9 × 9 cm piece of thin Styrofoam was hung on the inside of the opening with tape. The flap was cut larger than the opening and acted as a one-way entrance. We made a 10 × 10 cm screen window on the opposite side of the entrance for ventilation. A wet sponge was placed in the bottom of the container to minimize dehydration of trapped animals. Water and stones were also placed in each trap to provide refuges for animals and to stabilize the trap. A drift fence 60 cm high and 5 m long was constructed from nylon fabric. The bottom of the fence was buried 5 cm in the ground, and the fence was supported with stakes. A single trap was placed at each end of the fence. Each trap was buried 8 cm in the ground so the bottom of the entrance was flush with the ground. A wire mesh funnel was placed in front of the entrance to direct frogs from the drift fence into the trap. On each survey night, the trap was opened at 1900 hr and closed at 0700 hr the next morning, at which time trapped animals were identified and released.

### STATISTICAL ANALYSES

We performed statistical analyses using SAS (SAS Institute Inc., 1996). We used Pearson correlation coefficients to assess the relationship between the total number of species detected on a night of survey and weather variables. Data for all four sites were combined to give the total number of species detected on a night of survey, and data were log transformed to meet the parametric assumption of normality. In addition, a stepwise regression to determine the relative importance of each meteorological variable in predicting total number of species detected. We used one-way ANOVA to com-

pare the effectiveness between line transect sampling and other methods (automated recording system and trap methods combined) by testing the number of anuran species detected in each survey. We used the chi-squared test to examine the effectiveness of sampling methods among habitats.

RESULTS

SENSITIVITY OF THE SAMPLING TECHNIQUES

We conducted 27 fortnightly surveys at each site from July 2000 to July 2001 (Table 1). We completed all 108 line-transect surveys (27 days × 4 habitats) without significant problems or interruption. The automated

recorders had a failure rate of 6% (13/216), whereas traps failed only 1% of the time (1/108).

We detected 22 frog species belonging to five families (Table 1). Except for *Hyla chinensis*, *Buergeria japonica*, and *Microhyla ornata*, we detected other species at least six times during the 27 surveys. *Rana kuhlii*, *Rana latouchi*, and *Rana swinhoana* were found during every survey.

We detected 22 species of frogs using nocturnal line transects, with an average of 12.3±3.2 (range 7-17) species per survey. Line-transect sampling was more effective at detecting bufonids, microhylids, and ranids than other methods (Table 1; Fig. 1).

TABLE 1. The frequency of anuran species detected in 27 surveys by nocturnal line-transect sampling (L), automated recording system (R), and side-flap pail-trap (T) methods in a temporary pond, permanent pond, ephemeral stream, and permanent marsh in Taiwan. \*We could not differentiate between calls of *M. heymonsi* and *M. ornata*. We assumed most calls recorded were by *M. heymonsi*, because line-transect sampling revealed that *M. heymonsi* outnumbered *M. ornata* by 973 to 3.

	Temporary pond			Permanent pond			Ephemeral stream			Permanent marsh			Total Species Detected		
	L	R	T	L	R	T	L	R	T	L	R	T	L	R	T
BUFONIDAE															
<i>Bufo bankorensis</i>	11			4			11	1	1	23		3	24	1	4
<i>Bufo melanostictus</i>	5	1								1	1		6	2	0
HYLIDAE															
<i>Hyla chinensis</i>	1	1		1									1	2	0
RHACOPHORIDAE															
<i>Buergeria japonica</i>								1		1	2		1	3	0
<i>Buergeria robustus</i>	2	11		1	4		4	4		2			8	12	0
<i>Chirixalus eiffingeri</i>	6	9		8	16		17	25		15	24		23	27	0
<i>Chirixalus idiotocus</i>	18	19		9	15		7	5		14	18		18	22	0
<i>Polypedates megacephalus</i>	11	15		12	15					1	3		14	15	0
<i>Rhacophorus moltrechti</i>		3			2		8	8		22	20		23	19	0
<i>Rhacophorus taipeianus</i>	8	10		10	11		2	8		10	11		12	12	0
MICROHYLIDAE															
<i>Microhyla heymonsi</i>	13	13*	7	13	14*	1	5	5*		13	13*	2	15	14*	8
<i>Microhyla ornata</i>	1			2									3	0	0
RANIDAE															
<i>Rana adenopleura</i>	15	20		24	26	2	19	20	2	17	24	3	25	27	6
<i>Rana guentheri</i>	12	10	3		1								12	11	3
<i>Rana kuhlii</i>	2	2		5	8		25	12		27	17	7	27	18	7
<i>Rana latouchi</i>	22	18	1	22	21	8	25	20	11	26	16	2	27	27	15
<i>Rana limnocharis</i>	7	8	3	3	6	1				14	10		19	13	4
<i>Rana plancyi</i>	18	4		1									19	0	4
<i>Rana psaltes</i>					1					12	13	1	12	13	1
<i>Rana rugulosa</i>	4	6		3	6								6	9	0
<i>Rana sauteri</i>				2		1	8	5	1	2			9	5	2
<i>Rana swinhoana</i>							27	27	4	6	18		27	27	4
Total													22	20	11

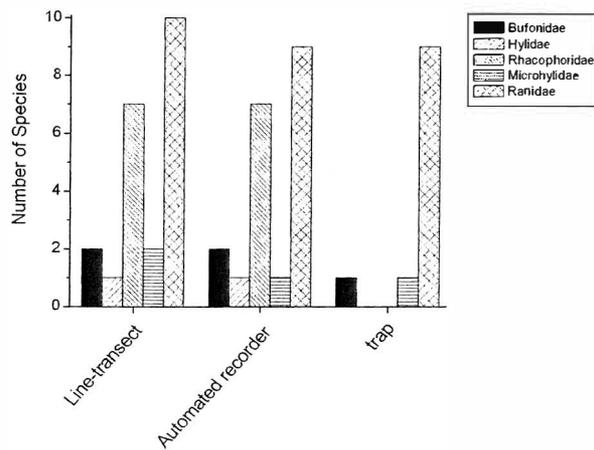


FIG. 1. The number of anuran species by families detected by line-transect sampling, automated recording system, and side-flap pail-traps methods in Taiwan.

We obtained recordings from 11,481 one-minute sampling intervals, and identified 21,503 frogs or groups of frogs. The results for *Microhyla heymonsi* and *M. ornata* were combined because it was difficult to distinguish their calls reliably. The automated recorders detected 20 species, with an average of  $10.4 \pm 3.5$  (range 1-12) species per survey. *Chirixalus eiffingeri*, *Rana adenoplura*, *R. latouchi*, and *R. swinhoana* were detected during every survey. The recorders did not pick up *Rana plancyi*, a species with a weak call, even though this frog was abundant at our study sites. Automated recorders were marginally more effective at detecting hylids and rhacophorids than line transects. The traps did not capture frogs from either of these two families (Table 1).

The traps captured only 11 frog species, with an average of  $2.1 \pm 1.5$  (range 0-3) species per survey. This technique was the least effective of all. Aside from *R. latouchi*, no anurans were detected more than 10 times during the 27 surveys, and the traps did not detect any arboreal species (e.g. rhacophorids and *H. chinensis*; Table 1; Fig. 1).

Each survey technique detected at least 60% of its cumulative total species during the first survey, and reached 90% after only 7-10 surveys (Fig. 2). The rate at which species accumulated was influenced by both season and weather. All three sampling methods recorded the most species between April and August, and the fewest between October and January. Weather had a significant influence on the number of species detected

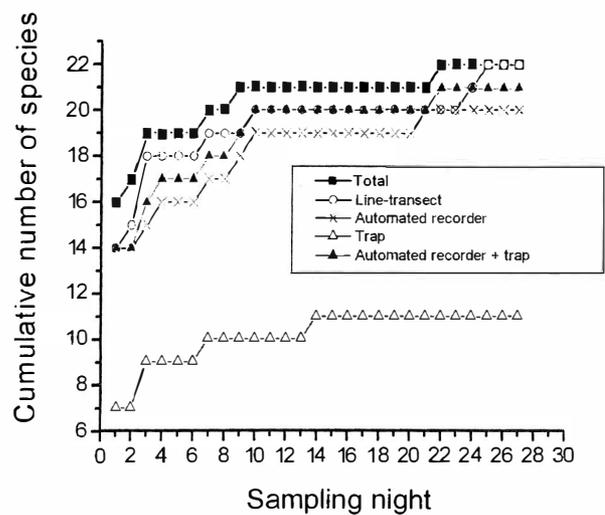


FIG. 2. Cumulative species detected by three sampling methods individually, the sum of three sampling methods, and the sum of automated recording system and side-flap pail-traps as a function of the number of survey. The number of survey corresponds to following date: 1=2000/7/5; 2=7/19; 3=8/2; 4=8/16; 5=8/31; 6=9/15; 7=9/30; 8=10/14; 9=10/28; 10=11/11; 11=11/25; 12=12/9; 13=12/21; 14=2001/1/7; 15=1/19; 16=2/1; 17=2/17; 18=3/3; 19=3/17; 20=3/31; 21=4/14; 22=4/28; 23=5/12; 24=5/26; 25=6/9; 26=6/23; 27=2001/7/7.

with each sampling technique (Table 3). Pearson correlation analyses revealed that cumulative rainfall for the four days prior to survey and air temperature one day post-survey were correlated significantly to the number of species detected for all three methods. Results of stepwise regressions revealed that the total number of species detected was influenced significantly only by cumulative rainfall for the four days prior to survey in line-transect sampling and side-flap pail-trap methods (Table 3), whereas the respective variables was influenced significantly only by air temperature in automated recording system.

COMPARISON OF SAMPLING TECHNIQUES

Detection of frogs was dependent on sampling technique (Fig. 2). The difference between line-transect sampling and automated recorders and traps combined was not significant (ANOVA,  $F_{1,52}=0.4$ ,  $P=0.528$ ). Line-transect sampling recorded the largest number of species at the temporary pond, ephemeral stream, and permanent marsh. Automated recorders detected the most species at the permanent pond. Traps were the least

TABLE 2. The number of frog species detected in four different habitats in Taiwan using three different sampling methods.

	Sampling sites			
	Temporary pond	Permanent pond	Ephemeral stream	Permanent marsh
Line-transect sampling	17	15	12	17
Automated recording system	15	15	13	14
Side-flap pail-trap	5	5	5	6

TABLE 3. Pearson correlation coefficients ( $r$ ) of correlation analyses and partial correlation ( $r^2$ ) of stepwise regressions between the number of species detected by three sampling methods and meteorological variables. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Weather variables	Pearson ( $r$ )	Partial $r^2$
<b>LINE-TRANSECT SAMPLING METHOD</b>		
Air temperature	0.43*	
Maximum air temperature	0.27	
Minimum air temperature	0.35	
Mean air temperature - four days pre-survey	0.34	
Air temperature - one day post-survey	0.47**	
Relative humidity	-0.41*	0.10*
Rainfall	0.29	
Total rainfall - four days pre-survey	0.62***	0.39***
Rainfall - one day post-survey	0.27	
	$P=0.0005$	
<b>AUTOMATED RECORDING SYSTEM</b>		
Air temperature	0.35	
Maximum air temperature	0.30	
Minimum air temperature	0.24	
Mean air temperature - four days pre-survey	0.27	
Air temperature - one day post-survey	0.42*	
Relative humidity	-0.36	0.07
Rainfall	0.26	
Total rainfall - four days pre-survey	0.63***	0.39***
Rainfall - one day post-survey	0.17	0.04
	$P=0.0004$	
<b>SIDE-FLAP PAIL-TRAP METHOD</b>		
Air temperature	0.51**	0.26**
Maximum air temperature	0.41*	
Minimum air temperature	0.46*	
Mean air temperature - four days pre-survey	0.33	0.06
Air temperature - one day post-survey	0.49*	
Relative humidity	-0.19	
Rainfall	0.19	
Total rainfall - four days pre-survey	0.46*	0.10
Rainfall - one day post-survey	0.10	0.09
	$P=0.0004$	

effective, capturing only one-third of species found in each site (Table 2). Despite these differences, there were no statistically significant differences in sampling techniques among habitats ( $\chi^2=0.50$ ,  $df=6$ ,  $P=0.99$ ).

DISCUSSION

Taiwan contains 29 species of anurans belonging to five families (Zhao & Alder, 1993; Lue *et al.*, 1999). Our surveys detected 22 species at our study area in Lien Hua Chih, which is seven more species than were found during an earlier study (Lu & Lin, 1995). In addition, this is the largest number of anuran species ever recorded at a single locality in Taiwan. Our success in detecting a wide range of species in an area with such high diversity indicates that our techniques are well suited for monitoring programs in Taiwan, and are likely to be useful in other tropical or subtropical regions as well.

Nocturnal line-transect sampling was the most effective method for detecting anurans in our study area, consistent with previous studies (Berrill *et al.*, 1992; Pearman *et al.*, 1995; Parris, 1999; Parris *et al.*, 1999). Past research has also shown that amphibian surveys are most effective during the warm, wet season, when most anurans are active (Aichinger, 1987; Bertoluci, 1998). In our study, the line-transect method was particularly effective during the wet season (March to June), when we detected 91% of the species at our study site. Some species can easily be missed, however. Because each survey took <1 hr at each site, species with small populations or with brief periods of activity could be overlooked. In Taiwan, this is particularly true for *H. chinensis*, *Buer. japonica*, *Buergeria robustus*, and *Rana rugulosa*.

Automated recorders were most effective at detecting species with loud calls. Nine of the most commonly

detected species fit this description (*H. chinensis*, *Buer. japonica*, *Buer. robustus*, *C. eiffingeri*, *Chirixalus idiootocus*, *Polypedates megacephalus*, *R. adenopleura*, *Rana psaltes*, and *R. rugulosa*). Recorders worked well at detecting *Microhyla* due to their loud, continuous choruses, but it was not possible to distinguish reliably between *M. ornata* and *M. heymonsi* due to the similarity of their calls. We did not record species that produce weak calls, even when the species was abundant (e.g., *R. plancyi*). In habitats with high levels of ambient noise, such as running water or nearby traffic, it would be more difficult to detect the calls of species with quiet vocalizations (e.g., *Bufo bankorensis*). Despite these weaknesses, the use of automated recorders was the second most effective technique, detecting 95% of the species at our study site. In Australia, Parris *et al.* (1999) used a similar technique and detected only 71% of the known species. The lower level of detection was probably due to differences in call characteristics in the local anuran communities.

In some situations, automated recorders had a distinct advantage over nocturnal line transects. Only automated recorders detected some species at the ephemeral stream (*Buer. japonica*), the temporary pond (*Rhacophorus moltrechti*), and the permanent pond (*H. chinensis*, *Rana guentheri* and *Rhaco. moltrechti*, Table 1). This is probably because calls were recorded throughout the night and species were detected that call only late at night, are secretive, or are relatively rare. In contrast, nocturnal line transects are typically conducted shortly after dark and seldom run throughout the night (Aichinger, 1987; Vandewalle *et al.*, 1996; Garcia-Rutledge & Narins, 2001). This might be important because Bridges & Dorcas (2000) reported that *Rana sphenoccephala* began to call after midnight; thus, nocturnal line transects would probably miss or underestimate the population size of this species or others with similar calling patterns.

Traps were the least effective of the three methods we tested, detecting only 50% of the species. Traps also had a relatively low capture rate of 31 animals/100 trap nights. Most trapping methods, including our side-flap pail-traps (Bury & Corn, 1987; Dodd, 1991; Greenberg *et al.*, 1994) captured mostly ground-dwelling species, even though funnel traps along drift fences have proven effective at catching arboreal anurans (Enge, 2001). In this study, the traps were particularly effective for ranids, capturing nine of the 10 local species. Nadorozny & Barr (1997) described the side-flap pail-trap method that we used, and they reported good success in capturing species that are strong jumpers (e.g., *Rana clamitans*, *R. pipiens*, and *R. sylvatica*). This result agrees with ours. Importantly, this type of trap was effective at capturing frogs of a wide range of sizes, from *M. heymonsi* (SVL 25 mm) to *R. guentheri* and *R. plancyi* (up to 80 mm SVL). Our study suggests that side-flap pail-traps can be much more effective than standard pitfall traps because the flap acts as a one-way

door and prevents escapes. We tested this idea prior to our surveys by placing eight species of frogs in traps and leaving them for three days, and no frogs escaped. In contrast, Parris *et al.* (1999) used standard pitfall traps and reported 0.56 animals/100 trap nights, and they detected only 14% of the total species found during the study. Most of the species studied by Parris *et al.* (1999) were treefrogs, species that could readily escape from standard pitfall traps.

The combined effectiveness of automated recorders and traps was equivalent to that of the line-transect sampling technique in detecting frogs; furthermore, even though the macro- and microenvironments of each habitat were different, our sampling methods worked consistently across all types of habitats. Thus, we believe that recorders and traps are a good substitute for nocturnal line transects. We recommend that automated recorders be considered as a viable sampling technique, especially in tropical or subtropical regions where nocturnal work is often dangerous, difficult, or remote. Even though automated recorders can be expensive initially (Corn *et al.*, 2000), the technique provides results that may not be obtainable otherwise, and allows for sampling over a longer time period than line transects. The combination of automated recorders and traps can document a high percentage of anurans in different community types. The availability of these two methods should encourage researchers to conduct amphibian monitoring even in remote areas that might otherwise be difficult to survey.

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#### REFERENCES

- Aichinger, M. (1987). Annual activity patterns of anurans in a seasonal neotropical environment. *Oecologia* **71**, 583-592.
- Barinaga, M. (1990). Where have all the froggies gone? *Science* **247**, 1033-1034.
- Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G. & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality

- associated with population declines in the rain forests of Australia and Central America. *Proceedings of National Academic of Science USA* **95**, 9031-9036.
- Berrill, M., Bertram, S., Bridhna, D., & Campbell, V. (1992). A comparison of three methods of monitoring frog populations. In *Declines in Canadian amphibian populations: Designing a national strategy*, 87-93. Bishop, C. A. and Pettit, K. E., (eds). Occasional Paper No. 76. Ottawa: Canadian Wildlife Service.
- Bertoluci, J. (1998). Annual patterns of breeding activity in Atlantic rainforest anurans. *Journal of Herpetology* **32**, 607-611.
- Bridges, A. S. & Dorcas, M. E. (2000). Temporal variation in anuran calling behavior: implications for surveys and monitoring programs. *Copeia* **2000**, 587-592.
- Bury, R. B. & Corn, P. S. (1987). Evaluation of pitfall trapping in northwestern forests: Trap arrays with drift fences. *Journal of Wildlife Management* **51**, 112-119.
- Corn, P. S., Muth, L. & Iko, W. M. (2000). A comparison in Colorado of three methods to monitor breeding amphibians. *Northwestern Naturalist* **81**, 22-30.
- Davidson, C., Shaffer, H. B., & Jennings, M. R. (2001). Declines of the California red-legged frog: spatial analysis of climate, UV-B, habitat and pesticides hypotheses. *Ecological Applications* **11**, 464-479.
- Davidson, C., Shaffer, H. B., & Jennings, M. R. (2002). Spatial tests of the pesticide drift, habitat destruction, UV-B and climate change hypotheses for California amphibian declines. *Conservation Biology* **16**, 1588-1601.
- Dodd, Jr. C. K. (1991). Drift fence-associated sampling bias of amphibians at a Florida sandhills temporary pond. *Journal of Herpetology* **25**, 296-301.
- Drost, C. A. & Fellers, G. M. (1996). Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, USA. *Conservation Biology* **10**, 414-425.
- Enge, K. M. (2001). The pitfalls of pitfall traps. *Journal of Herpetology* **35**, 467-478.
- Fellers, G. M. & Drost, C. A. (1993). Disappearance of the Cascades Frog, *Rana cascadae*, at the southern end of its range, California, USA. *Biological Conservation* **65**, 177-181.
- Fellers, G. M. & Freel, K. L. (1995). *A standardized protocol for surveying aquatic amphibians*. Technical Report NPS/WRUC/NRTR-95-01. National Biological Service, Cooperative Park Studies Unit. Davis: University of California.
- Garcia-Rutledge, E. J. & Narins, P. M. (2001). Shared acoustic resources in an old world frog community. *Herpetologica* **57**, 104-116.
- Greenberg, C. H., Neary, D. G., & Harris, L. D. (1994). A comparison of herpetofaunal sampling effectiveness of pitfall, single-ended, and double-ended funnel traps used with drift fences. *Journal of Herpetology* **28**, 319-324.
- Heyer W. R., McDiarmid, R. W., Donnelly, M. & Hayek, L. (1994). *Measuring and monitoring biological diversity. Standard methods for amphibians*. Washington: Smithsonian Institution Press.
- Lips, K. R. (1999). Mass mortality and population declines in anurans at an upland site in western Panama. *Conservation Biology* **13**, 117-125.
- Lue, C. Y., Tu, M. C. & Shang, G. (1999). *A field guide to the amphibians and reptiles of Taiwan*. Taipei: Great Nature Magazine Publisher.
- Lu, J.-F. & Lin, L.-H. (1995). Survey of amphibians in Nantou County. In *The biological Resources of Nantou County: a preliminary bioinventory report*, 33-53. Yen, J.-T. (ed.). Nantou: Taiwan Endemic Species Research Institute.
- Nadorozny, N. D. & Barr, E. D. (1997). Improving trapping success of amphibians using a side-flap pit-trap. *Herpetological Review* **28**, 193-194.
- Parris, K. M. (1999). Review: amphibian surveys in forests and woodlands. *Contemporary Herpetology*. **1999**, 1-16.
- Parris, K. M., Norton, W. T. & Cunningham, B. R. (1999). A comparison of techniques for sampling amphibians in the forests of south-east Queensland, Australia. *Herpetologica* **55**, 271-283.
- Pearman, P. B., Velasco, A. M. & Lopez, A. (1995). Tropical amphibian monitoring: a comparison of methods for detecting inter-site variation in species composition. *Herpetologica* **51**, 325-337.
- SAS Institute, Inc. (1996). *SAS/STAT User's Guide*. Cary: SAS Institute.
- Vandewalle, T. J., Sutton, K. K. & Christiansen, J. L. (1996). *Pseudacris crucifer*, an Iowa case history of an amphibian call survey. *Herpetological Review* **27**, 183-185.
- Zhao, E. M. & Adler., K. (1993). *Herpetology of China*. New York: Society for the Study of Amphibians and Reptiles.



## THE BOX TURTLE GENUS *TERRAPENE* (TESTUDINES: EMYDIDAE) IN THE MIOCENE OF THE USA

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Middle and Late Miocene fossils of the box turtle genus *Terrapene* are reviewed. The oldest known *Terrapene* specimens originate from the Medial and Late Barstovian North American Land Mammal Ages of Nebraska. Two nuchal bones from the Egelhoff Local Fauna, Keya Paha County, Nebraska (Medial Barstovian Land Mammal Age, ca 14.5-13.0 million BP) and a left hyoplastron from the Stewart Quarry, Cherry County, Nebraska (Late Barstovian, ca 13.0-11.5 million BP) closely resemble recent *Terrapene ornata*. The presence of *T. ornata*-like box turtles in the Barstovian suggests that the extant species groups were already established by that time and that the genus *Terrapene* evolved distinctly before the Middle Miocene. An extinct subspecies of *T. ornata*, *T. o. longinsulae* Hay, 1908, is known from the Late Miocene (Clarendonian, ca 11.5-9.0 million BP) as well as from several Pliocene sites. It is unknown whether a Clarendonian *Terrapene* hyoplastron from the Ash Hollow Formation of Cherry County, Nebraska belongs to *T. o. longinsulae* or to another taxon. A nearly complete anterior plastral lobe from the Myers Farm Local Fauna, near Red Cloud, Webster County, Nebraska, from the Late Barstovian (ca 13.0-11.5 million BP) differs significantly from all other recent and fossil *Terrapene* taxa. This specimen serves as the holotype of a new extinct *Terrapene* species described herein. It is of unclear relationships and shares some characters with *T. coahuila* and others with *T. nelsoni* and *T. ornata*. A left humerus from the WaKeeney Local Fauna, Trego County, Kansas (Middle to Late Clarendonian Land Mammal Age, ca 10.0-9.0 million BP) compares well with humeri of recent *Terrapene carolina* and is the oldest *Terrapene* that resembles extant *T. carolina*. The second oldest one is from the latest Miocene McGeehee Site, Alachua County, Florida (Early Hemphillian, ca 8.5 million BP) and perhaps represents a fossil taxon known as *T. carolina putnami* Hay, 1908.

*Key words:* Chelonia, fossil, Neogene, North America, palaeontology

### INTRODUCTION

The North American box turtles, genus *Terrapene*, are small (maximum carapacial length of recent taxa 200 mm), mostly terrestrial emydine turtles with a plastral hinge between the hyoplastron and the hypoplastron, allowing complete shell closure. The ability to close the shell entirely (like a box) is facilitated by a morphologically specialized tripartite scapula. The same structure is found in the closely related, hinged North American *Emydoidea blandingii* (Bramble, 1974), suggesting that the hinge and the segmented scapula are shared derived characters of *Emydoidea* and *Terrapene* (Gaffney & Meylan, 1988; Fritz, 2003). No other North American emydines have a plastral hinge and a segmented scapula, but in the Palearctic *Emys orbicularis*, a plastral hinge and a bipartite scapula occur (Bramble, 1974).

Box turtles form an essentially homogeneous group that has generally been taxonomically stable since the comprehensive studies of Milstead (1967, 1969) and Milstead & Tinkle (1967). *Terrapene* occurs only in the United States and Mexico. Four recent species are rec-

ognized, which are, with the exception of the aquatic *T. coahuila*, all mainly adapted to terrestrial habitats (Milstead, 1969; Ernst *et al.*, 1994; Minx, 1996; Dodd, 2001): (1) *Terrapene carolina* (Linnaeus, 1758) has four living subspecies restricted to the United States (*T. c. bauri*, *T. c. carolina*, *T. c. major*, and *T. c. triunguis*) and two restricted to Mexico (*T. c. mexicana* and *T. c. yucatanana*); (2) *Terrapene coahuila* Schmidt & Owens, 1944 is monotypic and exists only as an isolated population in Coahuila, Mexico; (3) *Terrapene nelsoni* Stejneger, 1925 has two living subspecies, both restricted to Mexico (*T. n. klauberi* and *T. n. nelsoni*); (4) *Terrapene ornata* (Agassiz, 1857) has two living subspecies, one (*T. o. luteola*) occurring both in the United States and Mexico, and one (*T. o. ornata*) only in the United States. However, recently it was suggested that the allopatric Mexican subspecies of *Terrapene carolina* might represent distinct species (Stephens & Wiens, 2003), *Terrapene mexicana* and *T. yucatanana*.

Two species groups, the "carolina group" and the "ornata group", are recognized (e. g. Milstead, 1967, 1969; Milstead & Tinkle, 1967; Minx, 1996; Dodd, 2001). Characters used to define the two groups are summarized in Milstead & Tinkle (1967), Milstead (1969), and Dodd (2001). The carolina group consists of *T. carolina* and *T. coahuila* and the ornata group of

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*T. ornata* and *T. nelsoni*. In each group, one extinct taxon is known which is treated traditionally as a subspecies. *Terrapene carolina putnami* Hay, 1908 ranged from a very large to a huge size (more than 300 mm shell length, Holman, 1965; Milstead, 1969; Dodd, 2001). It is otherwise quite similar to the extant *T. c. major*. Therefore, Auffenberg (1958) and Milstead (1969) speculated that *T. c. putnami* was the direct predecessor of *T. c. major*, which was influenced and modified by intergradation with other subspecies. *T. c. putnami* occurred from the Miocene-Pliocene boundary (Late Hemphillian, ca 5.5-4.5 million years BP) to the end of the Pleistocene (latest Rancholabrean, ca 10,000 years BP, Auffenberg, 1958; Milstead, 1969; Holman & Clausen, 1984). Also an Early Hemphillian find (ca 8.5 million years BP) has been tentatively identified as *T. c. putnami* (Milstead, 1969). *Terrapene ornata longinsulae* Hay, 1908 is known from the Late Miocene (Clarendonian, ca 11.5-9.0 million years BP) as well as from several Pliocene sites (Milstead, 1969). It differs from the extant *Terrapene ornata* subspecies by the low angle of elevation of the first central scute and the low elevation of the third central scute, the lack of rugosity of the carapacial scutes and non-flaring and non-emarginated marginal scutes (Milstead, 1969). It is beyond the scope of the present paper to discuss the advantages and problems of assigning subspecific rank to fossil taxa.

Other *Terrapene* fossils have been described as extinct species (e. g. Hay, 1908*a,b*, 1916; see the review in Dodd, 2001); but these, often fragmentary remains were later assigned to modern species (Auffenberg, 1958; Milstead, 1969). A recent morphometric study of the Pleistocene taxon *Terrapene innoxia* Hay, 1916 (relegated to the synonymy of *Terrapene carolina bauri* by Auffenberg, 1958) was done by Ernst *et al.* (1998). These authors suggested that the referral of *T. innoxia* to *T. c. bauri* is in doubt, and that further analysis of variation within *T. c. bauri* and *T. c. major* will be necessary to find out whether *T. innoxia* warrants taxonomic recognition. We feel, that it is highly probable that *T. innoxia* represents an intergrade population between modern subspecies of *T. carolina*.

In the present paper we deal with the scanty fossil material from the Middle and Late Miocene of Nebraska (Barstovian) and Kansas (Clarendonian) which was described in part earlier by Holman (1975, 1987) and Holman & Corner (1985). The material from the Barstovian of northwest Nebraska represents the earliest *Terrapene* known. Further, we review other Miocene *Terrapene*.

## LOCALITIES, MATERIALS AND METHODS

### THE FOSSIL LOCALITIES

We follow the concept that the Miocene extends from about 24 million to about 5 million years before the present (abbreviated here as BP). The Miocene in North

America begins in the later part of the Arikareean North American Land Mammal Age (NALMA) and continues to near the end of the Hemphillian NALMA. These Land Mammal Ages are as follow (Hulbert, 2001) [older to younger from bottom to top]:

Hemphillian	9.0-5.5 million BP
Clarendonian	11.5-9.0 million BP
Barstovian	16.0-11.5 million BP
Hemingfordian	20.0-16.0 million BP
Late Arikareean	24.0-20.0 million BP

The Barstovian NALMA that we will be mainly dealing with here has been subdivided into early, medial, and late portions (Voorhies, 1990):

Late Barstovian	13.0-11.5 million BP
Medial Barstovian	14.5-13.0 million BP
Early Barstovian	16.0-14.5 million BP

*The Medial Barstovian Site.* The Egelhoff Local Fauna (University of Nebraska State Museum, UNSM Collecting Locality Kp-101), also called the Egelhoff Quarry by Voorhies (1990), occurs in a road-cut north of the Niobrara River in Keya Paha County in northwest Nebraska. It is in the lower part of the Valentine Formation. Fossil material from this horizon was for years considered to overlap the Miocene-Pliocene boundary, but now is placed in the Middle Miocene in the Medial Barstovian NALMA (Voorhies, 1990). The upper fossiliferous unit of the Egelhoff Local Fauna is composed of unconsolidated, crossbedded sand with fine gravel near the base. Bulk screening (sieving) of the fine gravel lenses has yielded the great majority of the Egelhoff fossils and produced the *Terrapene* nuchal bones discussed below. Morris F. Skinner of Ainsworth, Nebraska discovered the site in 1964 and since then the site has been sampled by several institutions, including the Michigan State University Museum field parties which collected the *Terrapene* material in the interval between 1974 and 1978. Holman (1987) discussed previous herpetological studies at this site and Voorhies (1990) provided an expanded list of amphibians and reptiles.

*The Late Barstovian Sites.* The Myers Farm Local Fauna Quarry (UNSM Collecting Locality Wt-15A) lies in Webster County in southeastern Nebraska on the property of the Gary Myers family of Red Cloud. The site was discovered and studied by Corner (1976, 1977) who determined the Myers Local Fauna is stratigraphically equivalent to the type Valentine Quarry (Railroad Quarry) of north-central Nebraska. It is thus younger than the Egelhoff Local Fauna above and represents the Late Barstovian Land Mammal Age.

The Stewart Quarry (UNSM Coll. Loc. Cr 150) is a relatively new site in Cherry County, north-central Nebraska. Field crews from University of Nebraska State Museum have collected from this site since the early-

middle 1990s. This site also represents the Late Barstovian Land Mammal Age (M. J. Voorhies, pers. comm.).

*The Clarendonian Site.* The WaKeeney Local Fauna (University of Michigan Collecting Locality No. UM-K6-59) occurs on the Lowell Hillman Ranch in Trego County, in northwestern Kansas. The site was discovered by Lester F. Phillis in the early 1940's. Wilson (1968) published a comprehensive work on the vertebrates of the site. Later, J. A. Holman and field crews from the Michigan State University Museum (1969-1973) collected more than 100 tons of fossiliferous matrix from the site, adding to the known vertebrate fauna. Holman (1975) published a comprehensive report on the WaKeeney herpetofauna where he mentioned the *Terrapene* finding. Based on his analysis of the mammalian remains, Wilson (1968) assigned the WaKeeney Local Fauna to the Middle to Late Clarendonian Land Mammal Age (10.0-9.0 million BP).

#### SPECIMENS STUDIED

The *Terrapene* fossils described below are in the Michigan State University Museum Vertebrate Paleontological Collection, East Lansing (MSUVP) and in the University of Nebraska State Museum, Lincoln (UNSM). We compared the fossil specimens with 82 skeletons of recent *Terrapene* species, representing both extant species groups and most of the recognized recent taxa. Skeletal material of *Terrapene carolina yucatanica*, *T. ornata luteola* and *T. nelsoni klauberi* is extremely rare in museum collections (if existent at all) and was not available for comparison. The comparative recent material is from the holdings of the Michigan State University Museum, East Lansing (MSU), the Museum of Zoology (= Museum für Tierkunde) Dresden (MTD), and the University of Michigan Museum of Zoology, Ann Arbor (UMMZ) and is listed as follow:

*Terrapene coahuila* (n=5): MSU 3509-3510, 4108-4110; *Terrapene carolina bauri* (n=9): MSU 479, 487, 489, 521, 599, 629, 1306, 3061, 3825; *Terrapene carolina carolina* (n=22): MSU 510, 1401, 1696, 3454, 3941, 4020, 4115-4117, 4120, 4311, 4395, 12954, 13006, 13019, 14371, 14439-14440, 14442, MTD 8481, 42836, 43908; *Terrapene carolina major* (n=3): MSU 490, 3220, 3957; *Terrapene carolina mexicana* (n=1): MSU 3025; *Terrapene carolina triunguis* (n=16): MSU 1056, 4044, 4118-4119, 4349, 12958, 12963, 12966-12967, 12977, 12979, 13020, 14348-14350, 14362; *Terrapene nelsoni nelsoni* (n=1): UMMZ 128400; *Terrapene ornata ornata* (n=25): MSU 1563, 2091, 2815, 3023, 3107, 3147, 3149-3150, 3175, 3217, 3364, 3467, 3774, 4384, 12955, 12962, 12969, 12973, 12976, 13016-13018, MTD 43905-43906, 43334.

#### SYSTEMATIC PALAEOLOGY

*TERRAPENE* CF. *ORNATA* (AGASSIZ, 1857) (FIGS. 1 AND 2)

*Material.* Two nuchal bones, MSUVP 809A and 809B from the Egelhoff Local Fauna (UNSM Kp-101),

Keya Paha County, Nebraska; Middle Miocene (Medial Barstovian Land Mammal Age, ca 14.5-13.0 million BP). A left hyoplastron, UNSM 122393 from the Stewart Quarry (UNSM Cr-150), Cherry County, Nebraska; Middle Miocene (Late Barstovian Land Mammal Age, ca 13.0-11.5 million BP). A third nuchal bone (MSUVP 809C) from a juvenile specimen and a fragmentary right femur (MSUVP 809D) from the Egelhoff Local Fauna are much eroded and are only identifiable as *Terrapene*. All Egelhoff Local Fauna specimens were identified as *Terrapene* sp. indet. by Holman (1987).

*Identification.* The nuchal bones MSUVP 809A and 809B (Fig. 1) resemble *Terrapene ornata* and the single *T. n. nelsoni* (UMMZ 128400) studied in that they are markedly shorter and wider than in *T. carolina* and *T. coahuila*. They do not resemble nuchal bones of any other Nearctic turtle. The cervical scute impressions on the nuchal bones are similar to *T. ornata* and differ from *T. carolina*, *T. coahuila* and *T. n. nelsoni* in being clearly shorter and wider. The encroachment of the pleural sulci on the nuchal bone is more extensive in the fossil and in *T. ornata* than in *T. carolina*, *T. coahuila*, and *T. n. nelsoni*. These characters could not be observed in the Late Barstovian material described as a

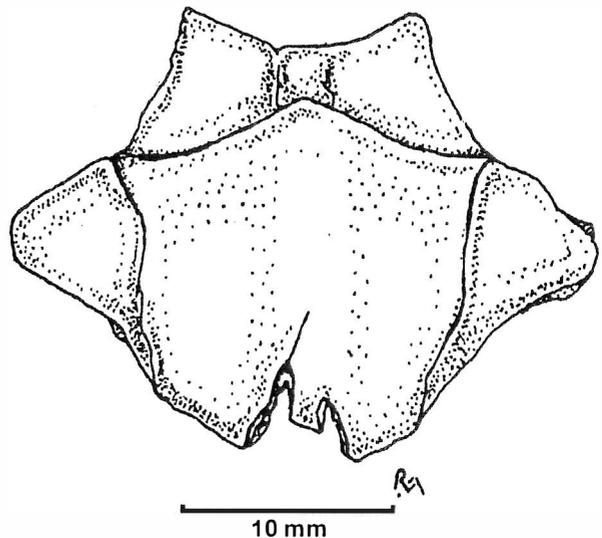


FIG. 1. *Terrapene* cf. *ornata* from the Egelhoff Local Fauna, Keya Paha County, Nebraska; Middle Miocene (Medial Barstovian Land Mammal Age, ca 14.5-13.0 million BP). Nuchal bone (MSUVP 809A). Drawing reproduced from Holman (1987).

new species below. The left hyoplastron UNSM 122393 (Fig. 2) resembles recent *T. ornata* and differs from all other species of *Terrapene* in its narrow humeral scute overlap and reduced articular surface for the bridge (Fig. 3). It resembles all other *Terrapene* species but differs from the Late Barstovian species in having the anterodorsal hinge line area extruded over the ventral hinge line area (Fig. 2).

*TERRAPENE* *CORNERI* N. SP. (FIG. 4)

*Holotype.* An anterior plastral lobe, complete except for a small piece of the right hyoplastron posterior to the

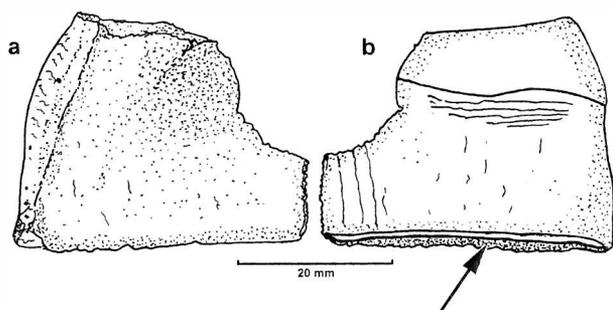


FIG. 2. *Terrapene* cf. *ornata* from the Stewart Quarry, Cherry County, Nebraska; Middle Miocene (Late Barstovian Land Mammal Age, ca 13.0-11.5 million BP). Left hyoplastron (UNSM 122393): (a) dorsal aspect, (b) ventral aspect. Arrow: anterodorsal hinge line area extruded over ventral hinge line area.

entoplastron, UNSM 21618. This specimen was identified as *Terrapene* sp. by Holman & Corner (1985).

**Locality and Horizon.** Myers Farm Local Fauna, near Red Cloud, Webster County, Nebraska (UNSM. Wt-15A); Middle Miocene (Late Barstovian Land Mammal Age, ca 13.0-11.5 million BP).

**Etymology.** Named in recognition of vertebrate palaeontologist R. George Corner who collected the holotype.

**Diagnosis.** *Terrapene corneri* resembles recent *Terrapene* and differs from all other emydine genera in having, in combination: (1) a kinetic plastron composed of a single grooved hinge with the pectoral-abdominal sulcus congruent with the hinge line, (2) plastral buttresses absent, and (3) the entoplastron encroached by both the gular-humeral and humeral-pectoral sulci. *Terrapene corneri* differs from all other living and fossil *Terrapene* taxa in having the following unique character combination: (a) presence of an epiplastral beak produced by two symmetrical gular notches, (b) very wide gular and humeral overlap, (c) notches in humeral and pectoral margin, and (d) anterodorsal border of hinge line area not extruded over anteroventral border of this area.

*T. corneri* differs from all *Terrapene* species except *T. coahuila* by the very wide gular and humeral overlap. An epiplastral beak resembling the condition in *T.*

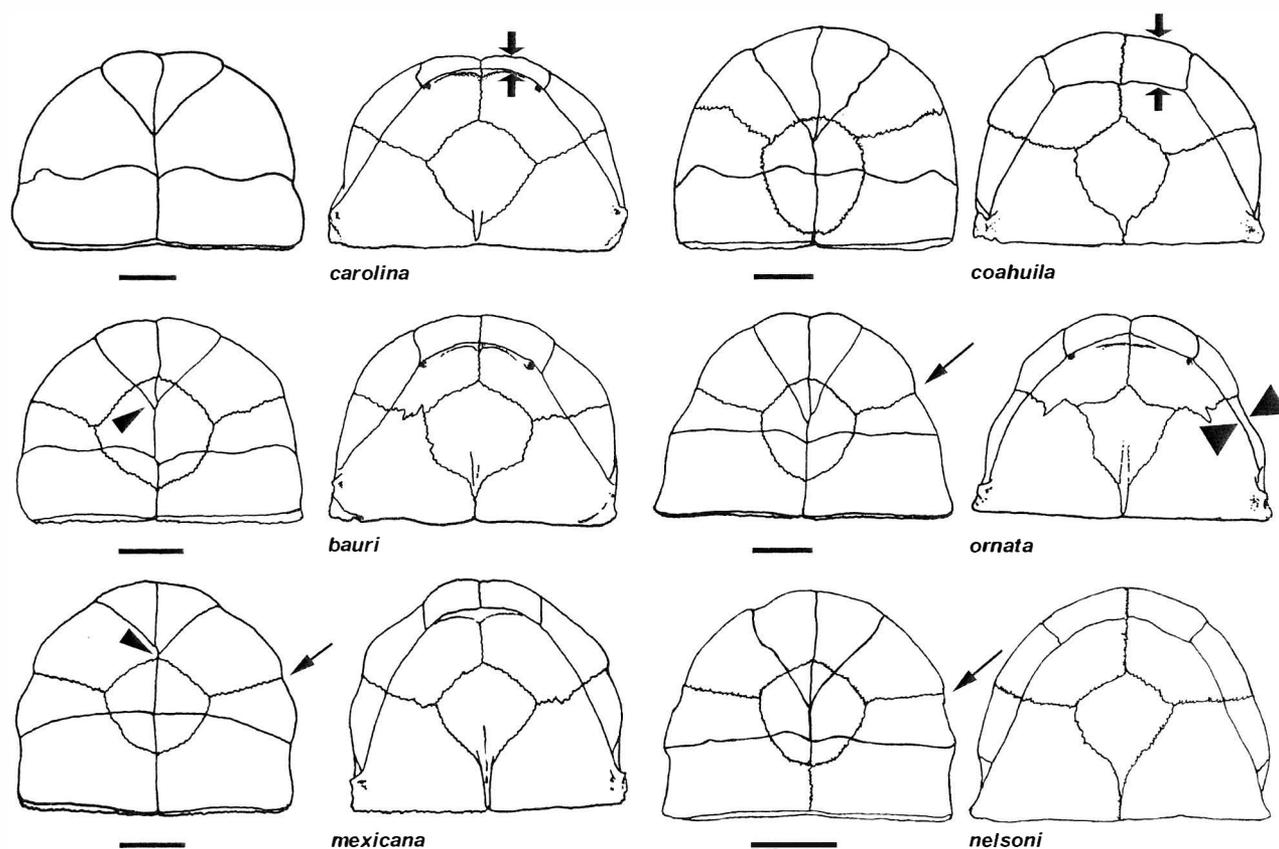


FIG. 3. Anterior plastral lobes of extant *Terrapene*. Ventral (left) and dorsal (right) aspects. Scale bars: 15 mm. *Terrapene carolina carolina*, specimen with externally entirely fused bony seams (MSU 1696, arrows: narrow gular overlap); *Terrapene carolina bauri* (MSU 3825, triangle: gular-humeral seams encroaching entoplastron); *Terrapene carolina mexicana* (MSU 3025, triangle: gular-humeral seams excluded from entoplastron, arrow: lateral notch at juncture of epiplastra and hyoplastra); *Terrapene coahuila* (MSU 4108, arrows: wide gular overlap); *Terrapene ornata ornata* (MSU 3217, arrow: lateral notch at juncture of epiplastra and hyoplastra, triangles: narrow humeral overlap); *Terrapene nelsoni nelsoni* (UMMZ 128400, arrow: lateral notch at juncture of epiplastra and hyoplastra).

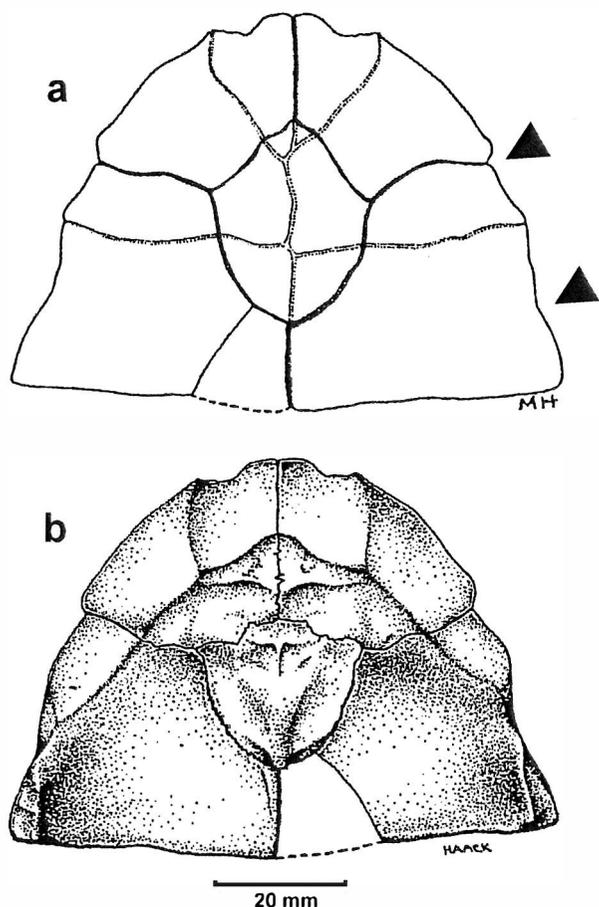


FIG. 4. *Terrapene corneri* n. sp., Myers Farm Local Fauna, near Red Cloud, Webster County, Nebraska; Middle Miocene (Late Barstovian Land Mammal Age, ca 13.0-11.5 million BP). Holotype, plastral forelobe (UNSM 21618): (a) ventral aspect, (b) dorsal aspect. Triangles: humeral and pectoral notches. The humeral notch is at the juncture of epiplastra and hyoplastra. Drawings reproduced from Holman & Corner (1985).

*corneri* may occur sporadically only in *T. coahuila*. *T. corneri* differs from *T. coahuila* significantly in the general shape of the plastral forelobe (*T. corneri*: narrow and anteriorly pointed, humeral and pectoral margin notched; *T. coahuila*: wide and rounded, humeral and pectoral margin not notched). *T. corneri* is similar to most *T. ornata* and the two sole *T. nelsoni* and *T. carolina mexicana* specimens studied and differs from all other *Terrapene* in having lateral notches in the anterior plastral lobe on the epiplastral and hyoplastral juncture, i. e., on the humeral margin. *T. ornata* and *T. nelsoni* differ from *T. corneri* by the ventrally more rounded entoplastron and their deep encroachment of the gular-humeral seams on the entoplastron. The single *T. carolina mexicana* studied differs from *T. corneri* in that the gular-humeral sulci are excluded from the entoplastron in *T. c. mexicana*.

**Description of the Holotype** (modified from Holman & Corner, 1985). The elongated holotype anterior plastral lobe implies that the plastron is ovoid in shape. The length of the forelobe is approximately 60 mm, the width 85 mm. It has a small, but distinct epiplastral beak

produced by the encroachment of two notches on the anterior edge of the epiplastron (margin of gular scutes). The anterior borders of these notches are slightly sinuate. There is also a lateral notch on each side of the anterior part of the lobe at the juncture of the epiplastral and hyoplastral bones (margin of humeral scutes) and at the medial hyoplastral margin (margin of pectoral scutes); these notches are very broadly V-shaped. In ventral view, the gular scute impressions form a triangle with the apex of the triangle directed posteriorly. The apex of the triangle enters the anterior part of the entoplastral bone. Each of the humeral scute impressions is roughly triangular with the apices of the triangles directed posteromedially. The median contacts between the gular and humeral scutes are about equal in length. The median contact between the humeral scutes extends about two-thirds the length of the entoplastron. The pectoral scutes are rectangular in shape. The anterior part of their median contact extends to about the posterior one-third of the length of the entoplastron. The epiplastral bones are very roughly triangular in shape, with the apices of these triangles directed anteromedially. The entoplastron is very roughly ovoid, with the anterior one-third forming a triangular apex anteriorly and the posterior two-thirds forming a roughly triangular apex extending posteriorly. About the anterior one-sixth of the bone is encroached by the gular-humeral sulci which are directed anteromedially; and about the posterior one-third of the bone is encroached by the humeral-abdominal sulci which are at right angles to the median axis of the bone. The postero-medial corner of the right hyoplastron is broken just below the entoplastron. There is no dorsal projection of the hinge line over the ventral portion of the hinge line.

In dorsal (visceral) view, the shell bones have about the same general shape and relationships to one another, except that in this view the entoplastron is roughly triangular in shape with its apex directed posteriorly. This allows a median contact between the epiplastral bones for almost their entire lengths. The basal portion of a ventrally posteriorly projecting spike is present, the anterior portion of the spike obviously having been broken off during the breakage in the posterior part of the lobe. A shallow but well developed depression in the dorsal surface of each epiplastron corresponds to the area for the insertion of the cervico-plastral ligament (aponeural cavity). The raised areas covered by gular and humeral scute overlap in life are wide. The articulation of the bridge is very robust.

**Comparison with other taxa.** A comparison with plastral forelobes of recent *Terrapene* leads to an estimated carapacial length of approximately 150-160 mm for the *Terrapene corneri* holotype. This is a size typical for most recent *Terrapene* taxa. In old adults of recent *Terrapene* species the bony seams of the shell are barely discernible on the shell surface due to a synostotical fusion of the sutures. This is illustrated in Fig. 3 for a *Terrapene carolina carolina* specimen (MSU 1696). In the *T. corneri* holotype all sutures on the anterior

plastral lobe are well developed externally (Fig. 4), suggesting either a character state like most other emydeans (that may be interpreted as plesiomorphic) or a subadult or young adult specimen.

The holotype of *T. corneri* does not resemble what might be called a "characteristic" *Terrapene* anterior plastral lobe. Corner (1977) succinctly stated this in reference to UNSM 21618, "Fossil turtles are common throughout the Tertiary of the Great Plains; most can readily be assigned to modern genera but such is not the case for an unusual form collected from Early Valentinian deposits near Red Cloud (UNSM Coll. Loc. Wt-15A), Webster County, Nebraska".

Holman & Corner (1985) compared UNSM 21618 in detail with other taxa and presented convincing arguments for assigning it to *Terrapene*. We will not repeat their comparison with Asian box turtles (*Cuora*, family Geoemydidae) here, but wish to emphasize that in the emydean genera *Emys* and *Emydoidea*, the humeral-pectoral sulcus is either entirely excluded from or barely entering the posterior part of the entoplastron, whereas in *Terrapene* the area is markedly encroached by this sulcus (Holman & Corner, 1985; Holman & Fritz, 2001). According to Holman & Corner (1985), UNSM 21618 resembles the extant *T. coahuila* in two characters which were not observed in other recent species: (1) The very wide areas where the gular and humeral scutes overlap the dorsal surface of the plastron, and (2) the large bridge articulations of the posterolateral portion of each side of the anterior plastral lobe.

A comparison of the plastral forelobe of *T. corneri* with recent *Terrapene* species follows (Fig. 3). The general shape of the *T. corneri* forelobe resembles a few *T. ornata* in that it is narrow and anteriorly somewhat pointed. In most *T. ornata* and all other taxa, the plastral forelobe has a more rounded appearance. In *T. corneri* the plastral margin is notched on the humeral margin at the borderline between the epiplastra and hyoplastra. A further notch occurs on each side on the pectoral margin. The same character state is found in most *T. ornata*. In a *T. n. nelsoni* (UMMZ 128400) the condition is similar to *T. ornata* and *T. corneri*. The forelobe is not emarginated there in *T. coahuila* and is not or is only feebly emarginated in most *T. carolina*. However, in our sole *T. carolina mexicana* (MSU 3925) there is a notch at the epiplastral-hyoplastral juncture, like in *T. ornata*, *T. nelsoni*, and *T. corneri*. If the margin of the anterior lobe is notched in the other comparative material of *T. carolina*, the emarginations are confined to the seam between humeral and pectoral scutes, not to the borderline between epiplastra and hyoplastra. The entoplastron of *T. corneri* is elongated, and on the dorsal (visceral) side escutcheon-shaped. Ventrally, its anterior portion is somewhat acutely triangular; the gular-humeral seams encroach the entoplastron only moderately. In *T. carolina* the entoplastron is wide and generally broadly diamond-shaped in dorsal aspect. Ventrally, it is broadly octagonal to round and its anterior portion is only infrequently triangular; and if so, it is

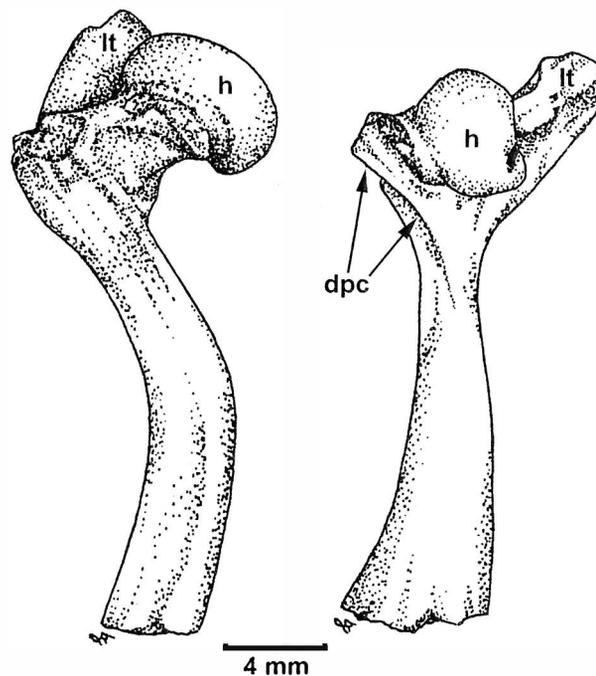


FIG. 5. *Terrapene* cf. *carolina* from the WaKeeney Local Fauna, Trego County, Kansas; Late Miocene (Middle to Late Clarendonian Land Mammal Age, ca 10.0-9.0 million BP). Left humerus (MSUVP 775), distal articulation destroyed. Abbreviations: h, head; lt, lateral tubercle (greater tuberosity); dpc, deltopectoral crest.

wide-angled. As in *T. corneri*, the gular-humeral seams only moderately encroach the entoplastron. In our *T. carolina mexicana* specimen, however, the gular-humeral sulci are excluded from the entoplastron. Compared with *T. carolina*, the entoplastron in *T. coahuila* may be somewhat more elongated. Otherwise the character states found *T. coahuila* are similar to *T. carolina*. In *T. ornata*, and the sole plastral forelobe of *T. n. nelsoni* studied by us, the entoplastron is rounded in ventral aspect and dorsally diamond-shaped. In contrast to *T. corneri*, *T. coahuila*, and *T. carolina*, the gular-humeral seams encroach deeply onto the entoplastron in *T. ornata* and *T. n. nelsoni*.

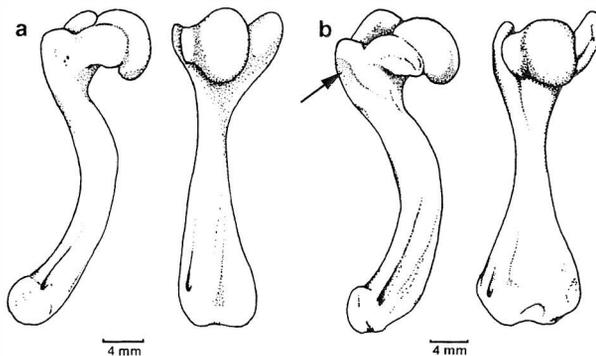


FIG. 6. Left humeri of (a) extant *Terrapene carolina carolina* (MSU 14439) and (b) extant *Terrapene ornata ornata* (MSU 3217). Arrow indicating the concavity formed by the deltopectoral crest in *T. ornata*.

Among the recent *Terrapene* taxa, *T. coahuila* resembles *T. corneri* in that one of five *T. coahuila* specimens studied has an epiplastral beak similar to *T. corneri*. Also, only *T. coahuila* has a wide gular and humeral overlap as observed in *T. corneri*. However, the general shape of the plastral forelobe differs significantly in both species: In *T. coahuila*, the forelobe is wide and rounded; in *T. corneri* it is narrow and anteriorly pointed (cf. Figs. 3 and 4). A further similarity between *T. corneri* and *T. coahuila* is the strong articulation of the bridge. On the other hand, the elongated shape of the *T. corneri* anterior plastral lobe is similar to some *T. ornata*, and the plastral notches at the borderline between epiplastra and hyoplastra in *T. corneri* resemble the condition found in *T. ornata*, *T. nelsoni*, and the sole *T. carolina mexicana* specimen studied. Thus, characters of several distinct recent taxa seem to be combined in *T. corneri*.

*TERRAPENE* CF. *CAROLINA* (LINNAEUS, 1758) (FIG. 5)

*Material.* A nearly complete left humerus, MSUVP 775 from the WaKeeney Local Fauna (UM-K6-59), Trego County, Kansas; Late Miocene (Middle to Late Clarendonian Land Mammal Age, ca 10.0-9.0 million BP).

*Identification.* This left humerus has been assigned to *Terrapene* cf. *carolina* by Holman (1975). Here we compare it with additional material of recent box turtles. The humerus is readily identifiable as *Terrapene* in having a compressed rather than a rounded head, a lateral tubercle that is directed more upward than outward, a deltopectoral crest that is directed more upward than inward, a moderately narrow excavation between the lateral tubercle and the deltopectoral crest, and a dorsoventrally bowed shaft. The humerus resembles the living species *T. carolina* and *T. coahuila* rather than *T. ornata* and *T. nelsoni* on the basis of what we believe is a strong character. In medial view in *T. carolina* and *T. coahuila*, the dorsolateral border of the deltopectoral crest is flat and the posterior portion of this crest is produced as a knob (also known as the minor tuberosity), whereas in *T. ornata* and *T. nelsoni* the dorsolateral border of the deltopectoral crest is concave and the posterior portion of the crest is produced upward as a knob (Fig. 6). The fossil is similar to *T. carolina* and *T. coahuila* in this character, but the crest is more expanded laterally than in either of the living species.

Parenthetically, we note that humeri of recent *T. carolina* and *T. coahuila* appear more slender than in *T. ornata* and *T. nelsoni* because in the latter the proximal and distal articulations are relatively broader. In *T. ornata* in particular, the lateral tubercle is stronger than in *T. carolina* and reflected somewhat inward. The fossil humerus resembles *T. carolina* in that its shaft appears more slender than in *T. ornata* and in that its greater tuberosity is not inwardly reflected.

The Clarendonian *Terrapene* hyoplastron from the Ash Hollow Formation of Cherry County, Nebraska

mentioned by Hutchison (1981) was unfortunately not available to us.

## DISCUSSION

We record the earliest known *Terrapene* as *T. cf. ornata* from the Middle Miocene (Medial Barstovian NALMA, ca 14.5-13.0 million BP) Egelhoff Site, in northwest Nebraska. The oldest record of *T. ornata* is of the extinct subspecies *T. ornata longinsulae* Hay, 1908 from the Late Miocene (Clarendonian, ca 11.5-9.0 million BP). Fossils from several Pliocene sites have been attributed to the same taxon (Milstead, 1969). The Egelhoff *T. cf. ornata* nuchals resemble *T. o. longinsulae* in representing small box turtles (maximum carapace length of *T. o. longinsulae* 125 mm in known specimens; Milstead, 1969). Based on a comparison with modern adult *T. o. ornata* from Trego County, Kansas, the fossil nuchal MSUVP 809A represents a box turtle with a straight line carapace length of approx. 95 mm and the fossil nuchal MSUVP 809B represents a specimen with a carapace length of approx. 75 mm. The left hyoplastron UNSM 122393 corresponds in size to extant *T. o. ornata* with a ca 100 mm straight line carapacial length. Obviously the two nuchal bones and the left hyoplastron do not represent an adequate sample, but the possibility remains that the Middle Miocene *T. cf. ornata* might have been even smaller than the Late Miocene *T. o. longinsulae*.

Milstead (1969) reports that *T. o. longinsulae* have smooth shells that differ from the rugose shells of modern *T. ornata*. The two Middle Miocene nuchals are both dorsally smooth and lack the distinct narrow keels that occur in modern *T. ornata* nuchals. This may indicate close relationships between the two forms. Nevertheless, we hesitate to assign the two nuchal bones to the extinct subspecies because of the lack of other shell elements.

Beside the left hyoplastron from the Stewart Quarry described herein, two other emydine turtles have been identified from this site, a *Chrysemys* species (Holman, 2002) and an extinct *Glyptemys* (Holman & Fritz, 2001), *G. valentinensis*. Other herpetofaunal remains have not been published from this site. It is assumed that the climate was similar during the deposition of the fauna at the Stewart Quarry as at other nearby Middle and Late Barstovian riparian faunas in north-central Nebraska which probably existed in a subtropical or at least equable, dry grassland habitat (Voorhies, 1990; J. A. Holman, unpubl.). Such conditions probably existed also at the Egelhoff Site in the Medial Barstovian.

The relationships of *Terrapene corneri* from the Middle Miocene (Late Barstovian) Myers Farm Local Fauna in southeastern Nebraska are uncertain. Some characters of *T. corneri* clearly resemble the recent *T. coahuila*, namely the very wide gular and humeral overlap and the strong hyoplastral bridge articulations. But it is also noteworthy that the entoplastron of *T. corneri* is elongated, and that among the recent species, only *T.*

*coahuila* has a somewhat elongate entoplastron. In addition, an epiplastral beak similar to that of *T. corneri* is presently known to occur sporadically only in *T. coahuila*. It has been often hypothesized that *T. coahuila* is the most ancestral form among the recent *Terrapene* species (Auffenberg, 1958; Legler, 1960; Burke *et al.*, 1996). Williams *et al.* (1960) and Bramble (1974) even suggested it was ancestral to the entire genus. However, new analyses of morphological (Minx, 1996) and molecular data sets (Feldman & Parham, 2002) have provided convincing evidence that *T. coahuila* is more closely related to *T. carolina* than to *T. nelsoni* or *T. ornata*, as earlier suggested by Milstead (1967, 1969) and Milstead & Tinkle (1967).

According to the morphological similarities mentioned above, one could speculate that *T. coahuila* is a descendent of *T. corneri*. If this is the case, *T. corneri* would represent the oldest record for the *carolina* group of *Terrapene*. On the other hand, notches at the sutures between the epiplastra and hyoplastra, as in *T. corneri*, do not occur in *T. coahuila* (and *T. carolina*, with the exception of *T. c. mexicana*) but they do in both species of the *ornata* group (*T. nelsoni*, *T. ornata*). Also the elongated shape of the *T. corneri* plastral forelobe is similar to some *T. ornata*. Thus, the Late Barstovian *T. corneri* appears to have morphological characters of both recent species groups. However, *T. corneri* is too young for being the last common ancestor of both groups as the Medial Barstovian Egelhoff Fauna fossils resemble the extant *T. ornata*. Therefore, we cannot exclude that *T. corneri* is a dead end lineage within *Terrapene*, perhaps representing the sister taxon of a clade containing the recent *carolina* and *ornata* groups.

Parenthetically, the considerable number of distinctive characters of our sole *T. carolina mexicana* anterior plastral lobe (MSU 3025) could support the recent suggestion of Stephens & Wiens (2003) that *mexicana* represents a distinct species rather than a subspecies of *T. carolina*. There is no obvious subspecies-specific variation among the specimens of other *T. carolina* subspecies studied by us. However, as we cannot exclude the possibility that the observed differences in our *mexicana* specimen are only individual aberrations, an investigation involving more osteological *mexicana* material is highly warranted.

In contrast to *T. cf. ornata* from the Middle Miocene (Medial and Late Barstovian) of northern Nebraska that existed in a semitropical or equable but dry grassland situation, *T. corneri* from the Late Barstovian Myers Farm Local Fauna of southeastern Nebraska lived in a moister, tropical to subtropical situation. The Myers Farm mammalian fauna differs from those of similar age in north-central Nebraska in consisting of fewer open-plains species and also has more southern species such as the artiodactyls *Prosynthetoceras* sp., *Longirostromeryx blicki* and *Pseudoparablastomeryx francesita*. Moreover, the perissodactyls also differ in that there is a decidedly higher frequency of low-crowned anchitherine horses (Corner, 1976) and tapirs (Schultz *et al.*,

1975) at Myers Farm than in the north-central Nebraska sites. The absence of the zapodid genus *Megasmithus*, the almost total lack of heteromyids, and an abundance of cricetid rodents are also distinct from north-central Nebraska where *Megasmithus* and various heteromyid species dominate the rodent fauna (Korth, 1979, 1980). Turning to the reptile fauna, one finds an undetermined trionychid, an undetermined emydid, *Terrapene corneri*, a giant and a smaller species of the tortoise, *Hesperotestudo*, as well as the alligator snapping turtle, *Macrochelys* sp. and two extinct boid snakes, seven colubrid snakes, an elapid and a viperid snake (Holman, 1977; Holman & Corner, 1985). It has long been suggested that the presence in fossil fauna of large land tortoises of the genus *Hesperotestudo* (long referred to as "*Geochelone*") suggests a climate with very mild winters where temperatures seldom if ever dropped to freezing (e. g. Hibbard, 1960). Considering the mammalian and herpetofauna of the Myers Farm locality, together, it seems likely that the climate in which all of these animals lived must have been at least as mild as that of the Gulf Coastal Plain of the United States today, and that winters were perhaps even milder.

The Clarendonian humerus from the WaKeeney Local Fauna (Trego County, Kansas), which is already discussed by Holman (1975), is the oldest *Terrapene* that we are aware of that resembles extant *T. carolina*. The second oldest one is from the latest Miocene McGeehee Site, Alachua County, Florida (Early Hemphillian, ca 8.5 million BP). It was tentatively assigned to *T. carolina putnami* (Milstead, 1969). Unfortunately, the taxonomic allocations of the Clarendonian *Terrapene* hyoplastron from the Ash Hollow Formation of Cherry County, Nebraska mentioned by Hutchison (1981) and of two Late Hemphillian nuchals from Knox County, Nebraska (Parmley, 1992) are unknown.

The *Terrapene* fossils discussed here emphasize that extant emydine turtle genera already existed in the Barstovian. Beside the *Terrapene* findings, the emydine genera *Emydoidea* (Hutchison, 1981; Holman, 1995, 2002) and *Glyptemys* (Holman & Fritz, 2001) are known from this Land Mammal Age. This suggests that the modern emydine turtle genera evolved distinctly earlier. Some of the Barstovian *Terrapene* fossils resemble *T. ornata*. Therefore, it is likely that the extant species groups of *Terrapene* were already established in that time and that the genus is distinctly older than the Middle Miocene.

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## REFERENCES

- Auffenberg, W. (1958). Fossil turtles of the genus, *Terrapene* in Florida. *Bulletin of the Florida State Museum* **3**, 53-92.
- Bramble, D. M. (1974). Emydid shell kinesis: biomechanics and evolution. *Copeia* **1974**, 707-727.
- Burke, R. L., Leuteritz, T. E. & Wolf, A. J. (1996). Phylogenetic relationships of emydine turtles. *Herpetologica* **52**, 572-584.
- Corner, R. G. (1976). *An early Valentinian vertebrate local fauna from southern Webster County, Nebraska*. Lincoln: M.S. Thesis, University of Nebraska.
- Corner, R. G. (1977). An unusual fossil turtle from the early Valentinian of Nebraska. *Proceedings of the Nebraska Academy of Sciences* **87**, 40.
- Dodd, C. K. (2001). *North American Box Turtles. A Natural History*. Norman: University of Oklahoma Press.
- Ernst, C. H., Lovich, J. E. & Barbour, R. W. (1994). *Turtles of the United States and Canada*. Washington, D. C. and London: Smithsonian Institution Press.
- Ernst, C. H., Wilgenbusch, J. C., Boucher, T. P. & Sekscienski, S. W. (1998). Morphometrics of the fossil box turtle, *Terrapene innoxia* Hay, 1916, from Florida. *Chelonian Conservation and Biology* **2**, 603-607.
- Feldman, C. R. & Parham, J. F. (2002). Molecular phylogenetics of emydine turtles: taxonomic revision and the evolution of shell kinesis. *Molecular Phylogenetics and Evolution* **22**, 388-398.
- Fritz, U. (2003). *Die Europäische Sumpfschildkröte*. Bielefeld: Laurenti Verlag.
- Gaffney, E. S. & Meylan, P. A. (1988). A phylogeny of turtles. In *The Phylogeny and Classification of the Tetrapods, Volume 1: Amphibians, Reptiles, Birds*, 157-219. Benton, M. J. (Ed). Oxford: Clarendon Press.
- Hay, O. P. (1908a). *The Fossil Turtles of North America*. Washington, D. C.: Carnegie Institute of Washington, Publication No. 75.
- Hay, O. P. (1908b). Descriptions of five species of North American fossil turtles, four of which are new. *Proceedings of the U.S. National Museum* **35**, 161-169.
- Hay, O. P. (1916). Descriptions of some Floridian fossil vertebrates, belonging mostly to the Pleistocene. *Florida State Geological Survey, Annual Report* **8**, 40-76.
- Hibbard, C. W. (1960). Pliocene and Pleistocene climates in North America. *Annual Report, Michigan Academy of Science, Arts and Letters* **62**, 5-30.
- Holman, J. A. (1965). A huge Pleistocene box turtle from Texas. *Quarterly Journal of the Florida Academy of Science* **28**, 345-238.
- Holman, J. A. (1975). Herpetofauna of the WaKeeney Local Fauna (Lower Pliocene: Clarendonian) of Trego County, Kansas. *University of Michigan Papers on Paleontology* **12**, 49-66.
- Holman, J. A. (1977). Upper Miocene snakes (Reptilia, Serpentes) from southeastern Nebraska. *Journal of Herpetology* **11**, 323-335.
- Holman, J. A. (1987). Herpetofauna of the Egelhoff Site (Miocene: Barstovian) of north-central Nebraska. *Journal of Vertebrate Paleontology* **7**, 109-120.
- Holman, J. A. (1995). A new species of *Emydoidea* (Reptilia: Testudines) from the Late Barstovian (Medial Miocene) of Cherry County, Nebraska. *Journal of Herpetology* **29**, 548-553.
- Holman, J. A. (2002). The status of *Emydoidea hutchisoni* Holman, 1995. *Michigan Academician* **34**, 393-394.
- Holman, J. A. & Clausen, C. (1984). Fossil vertebrates associated with Paleo-Indian artifact at Little Salt Spring, Florida. *Journal of Vertebrate Paleontology* **4**, 146-154.
- Holman, J. A. & Corner, R. G. (1985). A Miocene *Terrapene* (Testudines: Emydidae) and other Barstovian turtles from south-central Nebraska. *Herpetologica* **41**, 88-93.
- Holman, J. A. & Fritz, U. (2001). A new emydine species from the Middle Miocene (Barstovian) of Nebraska, USA with a new generic arrangement for the species of *Clemmys* sensu McDowell (1964). *Zoologische Abhandlungen, Museum für Tierkunde Dresden* **51**, 331-353.
- Hulbert, R. C. (2001). Florida fossil vertebrates. In *The Fossil Vertebrates of Florida*, 25-33. Hulbert, R. C. (Ed). Gainesville: University Press of Florida.
- Hutchison, J. H. (1981). *Emydoidea* (Emydidae, Testudines) from the Barstovian (Miocene) of Nebraska. *PaleoBios* **37**, 1-6.
- Korth, W. W. (1979). Geomyoid rodents from the Valentine Formation of Knox County, Nebraska. *Annals of Carnegie Museum* **48**, 287-310.
- Korth, W. W. (1980). Cricetid and zapodid rodents from the Valentine Formation of Knox County, Nebraska. *Annals of Carnegie Museum* **49**, 307-322.
- Legler, J. M. (1960). Natural history of the ornate box turtle, *Terrapene ornata ornata* Agassiz. *University of Kansas Publications, Museum of Natural History* **11**, 527-669.
- Milstead, W. W. (1967). Fossil box turtles (*Terrapene*) from central North America, and box turtles from eastern Mexico. *Copeia* **1967**, 168-179.
- Milstead, W. W. (1969). Studies on the evolution of box turtles (genus *Terrapene*). *Bulletin of the Florida State Museum* **14**, 1-113.
- Milstead, W. W. & Tinkle, D. W. (1967). *Terrapene* of western Mexico, with comments on the species groups in the genus. *Copeia* **1967**, 180-187.
- Minx, P. (1996). Phylogenetic relationships among the box turtles, genus *Terrapene*. *Herpetologica* **52**, 584-597.
- Parmley, D. (1992). Turtles of the Late Hemphillian (latest Miocene) of Knox County, Nebraska. *Texas Journal of Science* **44**, 339-348.
- Schultz, C. B., Martin, L. D. & Corner, R. G. (1975). Middle and late Cenozoic tapirs from Nebraska. *Bulletin of the University of Nebraska State Museum* **10**, 1-21.

- Stephens, P. R. & Wiens, J. J. (2003). Ecological diversification and phylogeny of emydid turtles. *Biological Journal of the Linnean Society* **79**, 577-610.
- Voorhies, M. R. (1990). *Vertebrate paleontology of the proposed Norden Reservoir area, Brown, Cherry, and Keya Paha Counties, Nebraska*. Lincoln: Division of Archaeological Research, Department of Anthropology, University of Nebraska.
- Williams, E. E., Smith, H. M. & Chrapliwy, P. S. (1960). Turtles and lizards from northern Mexico. *Transactions of the Illinois Academy of Sciences* **53**, 36-45.
- Wilson, R. L. (1968). Systematics and faunal analysis of a lower Pliocene assemblage from Trego County, Kansas. *Contributions from the Museum of Paleontology, University of Michigan* **22**, 75-126.

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## EXPERIMENTAL ANALYSIS OF GROUPING BEHAVIOUR IN CORDYLID LIZARDS

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Although the majority of cordylids are solitary, some species display prominent grouping behaviour. We tested whether limited shelter availability is a factor responsible for the grouping behaviour observed in *Cordylus cataphractus* and *C. macropholis*, using the solitary-living *C. polyzonus* as control. In an experimental setup, individuals of each species were provided with an excess of shelter sites and the observed patterns of shelter occupation were compared among the three species. *Cordylus cataphractus* consistently aggregated, occupying fewer shelters than its two congeners. Grouping behaviour in *C. cataphractus* in the wild is not the result of a limitation of refuge sites. In contrast, shelter occupation by *C. macropholis* and *C. polyzonus* was random, hence non-aggregative. Thus, although the aggregative behaviour observed in *C. macropholis* in its natural habitat might be influenced by limited shelter availability, other causal factors can not be excluded empirically.

**Key words:** *Cordylus cataphractus*, *Cordylus macropholis*, *Cordylus polyzonus*, Cordylidae, refuge availability

### INTRODUCTION

Although squamates are not generally group-living, temporary aggregations have been described in several species. In temperate zone species, these aggregations may occur during periods of reduced activity, for example during the winter as in certain *Eumeces*, *Urosaurus* and *Sceloporus* species (Neill, 1948; Worthington & Sabath, 1966; Weintraub, 1968; Ruby, 1977). Alternatively, such aggregations serve as a focal point for specific activities and have been termed accordingly. Sheltering (Hoofien, 1962; Myres & Eells, 1968) and basking (Hoofien, 1962; Myres & Eells, 1968) aggregations serve a strict thermoregulatory purpose, while mating (Pope, 1937; Hoofien, 1962) gestation (Graves & Duvall, 1993; Seburn, 1993) and nesting (Rand, 1967; Bock & Rand, 1989) aggregations are associated with reproductive behaviour. Feeding aggregations have also been described (Vitt, 1974; Arnold & Wassersug, 1978). Some authors have even linked the formation of temporary aggregations to possible anti-predatory (Vitt, 1974) and water conserving behaviour (Pope, 1937; Myres & Eells, 1968).

In contrast, few lizard species exhibit long-term or permanent aggregative behaviour. Long-term pair-fidelity has been reported in the viviparous skink, *Tiliqua rugosa* (Bull, 1994; Bull *et al.*, 1998). The Australian scincid genus *Egernia* also contains several gregarious species, with aggregations of individuals sharing rock crevices or burrows (Bull *et al.*, 2000). The gidgee skink (*Egernia stokesii*) forms stable social aggregations of up to 17 individuals that share the same rock crevice for several years (Main & Bull, 1996; Bull *et al.*, 2000).

The African cordylid genus *Cordylus* contains at least two species displaying aggregative behaviour (Mouton *et al.*, 1999), namely, the armadillo girdled lizard (*C. cataphractus*) and the large-scaled girdled lizard (*C. macropholis*). In both species, grouping is not restricted to any particular season or time of day, but occurs throughout the year. In addition, these groups are unlikely to represent family units as there is a high degree of intergroup movement (Visagie *et al.*, 2002; Nieuwoudt *et al.*, 2003a). *Cordylus cataphractus* presents one of the clearest manifestations of grouping behaviour observed within any lizard species to date. It is endemic to the arid and semi-arid areas along the west coast of South Africa (Mouton, 1988; Branch, 1998) where groups of up to 60 individuals may inhabit a single crevice in small sandstone outcrops (Visagie, 2001; Effenberger, 2004). Groups of two to six individuals are, however, the norm (Peers, 1930; Branch, 1998; Mouton *et al.*, 1999). Groups containing less than nine individuals usually include only one adult male plus several adult females and juveniles, but larger groups often include more than one adult male (Mouton *et al.*, 1999). *Cordylus macropholis* also occurs along the South African west coast. Although the succulent *Euphorbia caput-medusae* seems to be the preferred microhabitat of this species (Branch, 1998; Mouton *et al.*, 2000a; Nieuwoudt *et al.*, 2003a,b), it is not uncommon to find individuals sheltering in limestone cracks or alternative refuges (Branch, 1998). Aggregations of two to five (and occasionally up to 11) lizards can be found sheltering between the stems of a single succulent, and these aggregations rarely include more than one adult male (Mouton *et al.*, 2000a; Nieuwoudt *et al.*, 2003a,b).

Grouping behaviour in squamates is viewed as the result of either limited resource availability or mutual attraction of conspecifics, although these two causes are not necessarily mutually exclusive (Stamps, 1988; Graves & Duvall, 1995; Kearney *et al.*, 2001). In our

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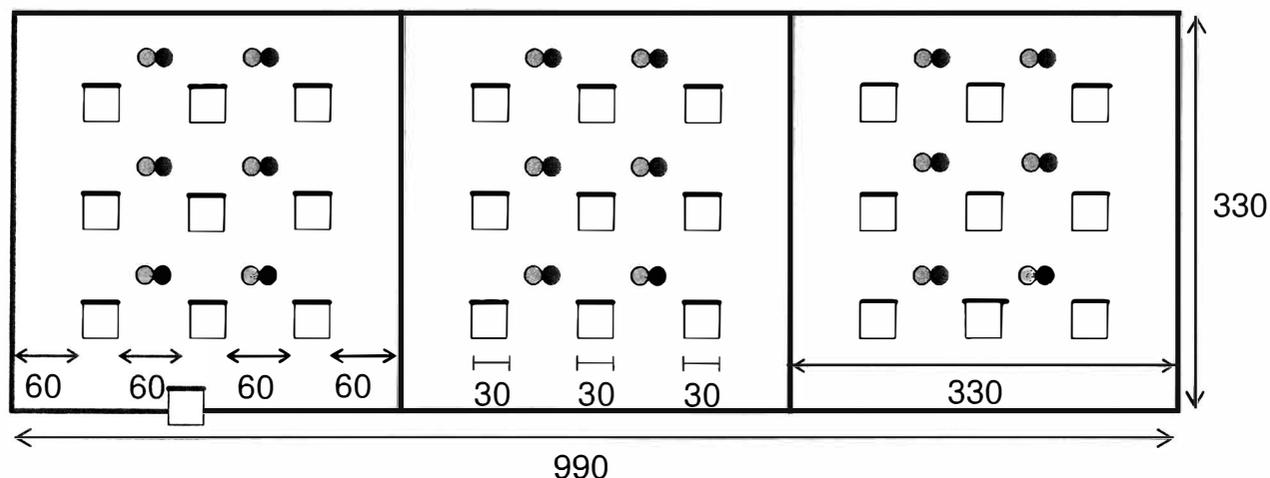


FIG. 1. Diagram of the experimental setup used to determine whether limited shelter availability influences aggregation in *Cordylus cataphractus*, *C. macropholis* and *C. polyzonus*. Enclosure walls are from asbestos sheets. Small squares represent individual shelters (30 × 30 cm) with darker lines representing the north-facing shelter entrance. Solid circles represent food dishes and open circles water dishes. All measurements are in centimeters.

study, we aimed to test the hypothesis that limited shelter availability is a factor inducing aggregative behaviour in *C. cataphractus* and *C. macropholis*. Over a large part of the range of *C. cataphractus*, specifically along the western coastal lowlands, rock outcrops appear to be a limited resource. Likewise, the distribution of *Euphorbia* plants, considered to be the preferred refuge of *C. macropholis*, is extremely patchy over most of this lizard's range.

Our approach consisted of exploring whether *C. cataphractus* and *C. macropholis* individuals continue to display aggregative behaviour when presented with an excess of shelters, while controlling for alternative factors which might prompt aggregation. Thus our null-hypothesis predicts that the lizards will not exhibit grouping behaviour when an excess of shelter sites are provided. Patterns of shelter occupation by these two species were compared to that obtained for a solitary-living congener, *C. polyzonus* (Branch 1998). This controls for any possible "Allee effect", i.e., where species display unnatural aggregations under experimental conditions in contrast to their field behaviour (Allee, 1931; Stamps, 1988). All three cordylids are territorial, sit-and-wait feeders (Cooper *et al.*, 1997; Mouton *et al.*, 2000a; Mouton *et al.*, 2000c; Effenberger & Mouton, 2003). Our experiment was conducted outside the mating season of these species (Flemming & Van Wyk, 1992; Mouton *et al.*, 2000a; Flemming & Mouton, 2002) to minimize the effects of territoriality and agonistic behaviour. Lizards of all three cordylids were collected and exposed to trials in identical sex ratios. Lastly, excess water and food supplies were distributed throughout the test arena to counter for the effects of patchy or limited availability of these resources.

## MATERIALS AND METHODS

### COLLECTION OF SPECIMENS

Lizards were collected during late November 1998 near Lambert's Bay, along the west coast of South Af-

rica and approximately 300 km north of the University of Stellenbosch where the experiments were conducted. Seven adult males and eight adult females of each species were collected using noosing techniques. Size at sexual maturity were taken as 95 mm for *C. cataphractus* (Mouton *et al.*, 1999; Flemming & Mouton, 2002), 58 mm for *C. macropholis* (Mouton *et al.*, 2000a) and 89 mm for *C. polyzonus* (Flemming & Van Wyk, 1992). Males were distinguished by the presence of large hemipenial bulges, broad heads, and the presence of a relative large number of generation glands (Van Wyk & Mouton, 1992). All lizards were released at their site of capture upon completion of the experiment.

### HOUSING OF SPECIMENS

Specimens were transported to the University of Stellenbosch where they were housed singly in glass terraria in a laboratory prior to experimentation. An arbitrary period of four weeks was allowed for acclimation to captive conditions. A laboratory window allowed exposure to the natural photoperiod. Room temperature was maintained at 29 °C during daytime, and was allowed to follow ambient temperature at night. Each terrarium was provided with newspaper substrate and shelter sites constructed from two ceramic tiles separated by two wooden strips. Water and food, primarily tenebrionid larvae, were available *ad libitum*.

### EXPERIMENTAL SETUP

An open-air experiment was conducted on the roof of a building on the university campus. This provided exposure to the natural photoperiod and weather conditions, and limited human interference. Three adjacent 3.30 m<sup>2</sup> enclosures were constructed using asbestos sheets held upright with bricks (Fig. 1). Crushed stone was added as substrate. Nine artificial shelters were spaced out in a symmetrical block pattern in each of the three enclosures. A shelter consisted of (top to bottom): a styrofoam cover (30 × 30 × 8 cm) to reduce radiation

heat from the sun; a plywood board (30 × 30 × 2 cm) as shelter cover; three plywood side strips (two 30 × 2 × 2 cm and one 26 × 2 × 2 cm) leaving one side open as a shelter entrance; and an asbestos base (30 × 30 × 0.2 cm). Crevice size was selected to be large enough to house all five lizards, but at the same time also small enough to ensure physical contact among lizards occupying a crevice. All shelter entrances were north facing. A brick was placed on top of each shelter to stabilize the structure. Only horizontal crevices were provided because of the preponderance of this type of crevice in the area where the lizards were collected (see also Mouton *et al.* 1999). One food and one water dish was placed in front of and between two shelter openings (Fig. 1).

#### EXPERIMENTAL PROCEDURE

We conducted three consecutive week-long trials (i.e. replicates), with species assigned randomly to one of the three enclosures at the onset of every trial. Five conspecific lizards were released at a single location within the designated enclosure of each species. Lizards were allowed to acclimate to conditions for a period of 24 hours. During the following seven days, the number of lizards residing under each shelter was recorded per enclosure at 20.00 hrs, when all activity for the day had ceased. Fresh water and food were also supplied at this time. On days exceeding 28 °C, the entire experimental area was irrigated at 12.00 hrs and additional drinking water was supplied. All shelters as well as the crushed stone substrate were thoroughly washed with water and cleaning agent between trials. Five novel lizards of each species were introduced into the experimental arena for every trial, in other words, a total of three groups and thus 15 lizards were tested per species. Two of these groups consisted of two males and three females, while the remaining group consisted of three males and two females. In the case of *C. cataphractus* and *C. macropholis*, these artificial groups never consisted of more than one member of the natural groups collected.

#### RESULTS

We explored whether individuals of the three species showed a tendency to aggregate in shelter sites at night. The test statistic was the number of shelters containing lizards on a given night, ranging between one (i.e. all five lizards under a single shelter) and five (i.e. each lizard sheltering individually). To detect grouping behaviour, the frequency distributions of observed scores for each species were compared to frequencies expected under the null hypothesis that lizards shelter randomly according to the urn or occupancy statistical model (Parzen, 1960). According to this model, the expected mean value is 4.01 occupied shelters. The three species displayed varying degrees of aggregation. *Cordylus cataphractus* used two to four shelters per night (Fig. 2), which is significantly less than expected under the occupancy model (Kolmogorov-Smirnov one-sample test,  $d_{max} = 11$ ,  $P < 0.01$ ). On all nights at least two individuals shared a shelter. The maximum number of lizards under

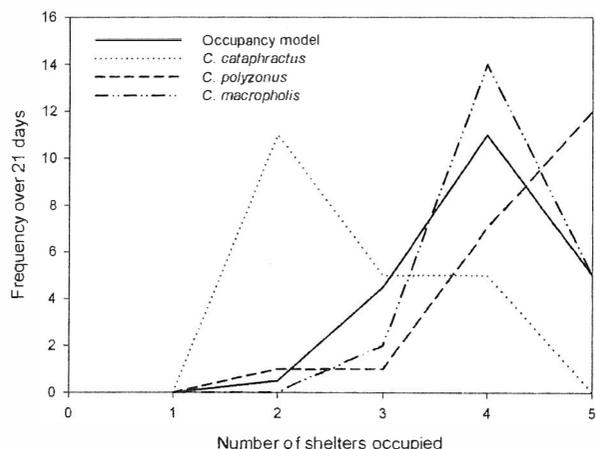


FIG. 2. Line graph depicting frequencies of shelter occupation by five *C. cataphractus* individuals, five *C. polyzonus* individuals, and five *C. macropholis* individuals for a period of 21 days – each day is considered a separate sample. The expected frequencies under the occupation statistical model (Parzen 1960) are also depicted.

a single shelter was four, which was observed on five occasions. There was no significant difference in the number of shelters used by *Cordylus macropholis* and the number of shelters expected to be used under the occupancy model (Kolmogorov-Smirnov one-sample test,  $d_{max} = 3$ ,  $P > 0.05$ ). Although the majority of lizards sheltered singly, on rather frequent occasions two lizards shared the same shelter. We never found more than two *C. macropholis* lizards under a single shelter. *Cordylus polyzonus* occupied significantly more shelters than expected under the occupancy model (Kolmogorov-Smirnov one-sample test,  $d_{max} = 7$ ,  $P < 0.05$ ), which suggests mutual avoidance of individuals. Lizards predominantly sheltered singly or in pairs, except for one occasion where three *C. polyzonus* individuals were found together (Fig. 2).

The former results suggest that *C. cataphractus* exhibited a significant tendency to aggregate when provided with excess shelters, whereas *C. macropholis* and *C. polyzonus* did not. However, our analyses assumed that the data for each group of lizards on consecutive nights represent independent samples. This may increase the probability of falsely rejecting the null-hypothesis. To avoid this, a Repeated Measurements ANOVA was used to compare the pattern of shelter occupation by *C. cataphractus* to that of *C. macropholis* and *C. polyzonus*, the species that did not show any tendency to aggregate. Since the RM Anova only tests for relative differences and provides no point of reference for randomness of distribution, the null hypothesis simply predicts no difference in observed distribution patterns. This analysis treated the number of shelters occupied on consecutive nights by each experimental group as dependent variables, while “time” (i.e. consecutive nights) served as the within-subjects factor and species as the between-subject factor. We detected no significant interaction effect between species and the seven consecutive trial nights ( $F_{12,36} = 0.845$ ,

$P > 0.6$ ). In other words, any differences in shelter occupation among species remained approximately constant over the experimental period. The number of shelters used by each species did not vary significantly among different nights ( $F_{6,36} = 0.270$ ,  $P > 0.2$ ). A significant difference was however detected in the number of shelters occupied among the three species ( $F_{2,6} = 8.166$ ,  $P < 0.05$ ). A *post hoc* Newman-Keuls test indicated no difference in shelter occupation between *C. polyzonus* and *C. macropholis*, but *C. cataphractus* used significantly fewer shelters than both these species.

In conjunction with the first test, this indicates that *Cordylus cataphractus* displays aggregative behaviour even if presented with an excess of shelter sites. *Cordylus macropholis* displayed random shelter occupancy as expected by the occupancy model. Shelter occupation by *C. polyzonus* did not differ significantly from that of *C. macropholis* according to the RM ANOVA, thus the mutual avoidance detected for this species in the occupancy test must be interpreted with caution.

#### DISCUSSION

A significant difference was apparent in the pattern of shelter occupation among our three test species. *Cordylus cataphractus* consistently occupied fewer shelters than *C. macropholis* and *C. polyzonus* did under identical experimental conditions. Since this grouping behaviour was additionally exhibited in the presence of an excess of shelter sites, our working hypothesis is rejected for this species: grouping behaviour in *C. cataphractus* is not the result of limited shelter availability. In contrast, *C. macropholis* displayed a pattern of shelter occupation that did not differ significantly from that of *C. polyzonus*. Our working hypothesis is accepted in this case, in other words, limited shelter availability can not be excluded as a factor contributing to the aggregative behaviour naturally observed in *C. macropholis*.

These findings are well supported by field studies. Of 134 *C. cataphractus* collected by Mouton *et al.* (1999) along the western coastal lowlands, 85 % of individuals were found in groups of two or more, illustrating the strong tendency to aggregate in the species. The distribution of *C. cataphractus* is not restricted to these coastal lowlands, where rocky outcrops are limited, but also occur inland and in more mountainous areas, where there is an abundance of crevices. Individuals nevertheless still aggregate in groups in the latter areas (Peers, 1930; personal observations), indicating mutual attraction of conspecifics. Furthermore, members of a group not only share the crevice as a nighttime refuge, but during daytime the entire group utilizes the rock containing the home crevice as an elevated look-out point from where foraging attempts are launched, despite the fact that this promotes intraspecific food

competition (Mouton *et al.*, 2000c). In the case of *C. macropholis*, field studies have shown the incidence of lizards sharing plants to be as high as 89 % (Mouton *et al.*, 2000a). Unlike *C. cataphractus*, however, these lizards displayed mutual avoidance when provided with an excess of shelter sites in our experiment. The distribution of *E. caput-medusae* is extremely patchy across the range of *C. macropholis* and the plants can be considered a limited resource, thus forcing lizards to co-inhabit the same plant (personal observations). The high incidence of one *C. polyzonus* lizard per shelter in our experiment also correlates with field observations, where individuals are predominantly encountered singly (Branch, 1998; personal observations).

*C. cataphractus* displays several unique physiological and behavioural characters, which may be associated with energy constraints brought about by the permanent group-living lifestyle of this sit-and-wait forager. These characters include low fecundity, with females giving birth to only one young per year as opposed to two to nine in other cordylids (Flemming & Mouton, 2002), an extremely low resting metabolic rate (Mouton *et al.*, 2000b), and extremely low activity levels during dry summer months (Visagie, 2001) when other cordylids remain active. No such unique characters have been encountered in *C. macropholis* as of yet, once again enforcing our hypothesis that aggregative behaviour in *C. macropholis* is the result of limited availability of refugia.

In light of our evidence that different driving forces are responsible for the grouping behaviour observed in *C. cataphractus* and *C. macropholis*, it becomes questionable whether aggregations of *C. macropholis* should be classified as social groupings. The dense spatial arrangement of *E. caput-medusae* stems allows limited visual contact between individuals and mutual avoidance can easily be maintained. On the other hand, *C. cataphractus* lizards are in constant visual contact and, to a lesser extent, physical contact within their rock crevices (personal observations). Lizards are forced to interact socially and a complex social structure is to be expected.

In conclusion, limited crevice availability can be eliminated as being responsible for the strong grouping tendency in *C. cataphractus*. It will be difficult to assess whether the unique characteristics of *C. cataphractus* are the cause of its group-living behaviour or a consequence thereof. In contrast, aggregative behaviour observed in *C. macropholis* might be an artifact of limited refuge availability and social structure in this species is potentially not as complex as in *C. cataphractus*. These two species provide ideal opportunities for further investigations into social structure among reptiles.

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## REFERENCES

- Allee, W. C. (1931). *Animal aggregations: a study in general sociology*. University of Chicago Press, Chicago.
- Arnold, S. J. & Wassersug, R. J. (1978). Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. *Ecology* **59**, 1014-1022.
- Bock, B. C. & Rand, A. S. (1989). Factors influencing nesting synchrony and hatching success at a green iguana nesting aggregation in Panama. *Copeia* **1989**, 978-986.
- Branch, W. R. (1998). *Field Guide to Snakes and Other Reptiles of Southern Africa*. Cape Town: Struik Publishers.
- Bull, C. M. (1994). Population dynamics and pair fidelity in sleepy lizards. In *Lizard Ecology: Historical and Experimental Perspectives*, 159-174. Vitt, L. J. and Pianka, E. R. (Eds). Princeton: Princeton University Press.
- Bull, C. M., Cooper, S. J. B. & Baghurst, B. C. (1998). Social monogamy and extra-pair fertilization in an Australian lizard, *Tiliqua rugosa*. *Behavioral Ecology and Sociobiology* **44**, 63-72.
- Bull, C. M., Griffin, C. L., Lanham, E. J. & Johnston, G. R. (2000). Recognition of pheromones from group members in a gregarious lizard, *Egernia stokesii*. *Journal of Herpetology* **34**, 92-99.
- Cooper, W. E. Jr., Whiting, M. J. & Van Wyk, J. H. (1997). Foraging modes of cordyliform lizards. *South African Journal of Zoology* **32**, 9-13.
- Effenberger, E. (2004). Social structure and spatial use in a group-living lizard, *Cordylus cataphractus*. Unpublished M. Sc. thesis, University of Stellenbosch, South Africa.
- Effenberger, E. & Mouton, P. le F. N. (2003). Social structure and spatial use in a group-living lizard, *Cordylus cataphractus*. *Proceedings of the Joint Conference of SASAQS & ZSSA, University of Cape Town, Cape Town, July 2003*. p. 36.
- Flemming, A. F. & Mouton, P. le F. N. (2002). Reproduction in a group-living lizard, *Cordylus cataphractus*, from South Africa. *Journal of Herpetology* **36**, 691-696.
- Flemming, A. F. & Van Wyk, J. H. (1992). The female reproductive cycle of the lizard *Cordylus p. polyzonus* (Sauria: Cordylidae) in the Southwestern Cape Province, South Africa. *Journal of Herpetology* **26**, 121-127.
- Graves, B. M. & Duvall, D. (1993). Reproduction, rookery use, and thermoregulation in free-ranging, pregnant *Crotalus v. viridis*. *Journal of Herpetology* **27**, 33-41.
- Graves, B. M. & Duvall, D. (1995). Aggregation of squamate reptiles associated with gestation, oviposition, and parturition. *Herpetological Monographs* **9**, 102-119.
- Hoofien, J. H. (1962). An unusual congregation of the gekkonid lizard *Tarentola annularis* (Geoffroy). *Herpetologica* **18**, 54-56.
- Kearney, M., Shine, R., Comber, S. & Pearson, D. (2001). Why do geckos group? An analysis of "social" aggregations in two species of Australian lizards. *Herpetologica* **57**, 411-422.
- Main, A. R. & Bull, M. (1996). Mother-offspring recognition in two Australian lizards, *Tiliqua rugosa* and *Egernia stokesii*. *Animal Behavior* **52**, 193-200.
- Mouton, P. le F. N. (1988). *Cordylus cataphractus*: species account. In *South African Red Data Book – Reptiles and Amphibians*, 158. Branch, W.R. (Ed). South African National Scientific Programmes Report No 151. Pretoria: Council for Scientific and Industrial Research.
- Mouton, P. le F. N., Flemming, A. F. & Kanga, E. M. (1999). Grouping behaviour, tail-biting behaviour, and sexual dimorphism in the armadillo lizard (*Cordylus cataphractus*) from South Africa. *Journal of Zoology, London* **249**, 1-10.
- Mouton, P. le F. N., Flemming, A. F. & Nieuwoudt, C. J. (2000a). Sexual dimorphism and sex ratio in a terrestrial girdled lizard, *Cordylus macropholis*. *Journal of Herpetology* **34**, 379-386.
- Mouton, P. le F. N., Fourie, D. & Flemming, A. F. (2000b). Oxygen consumption in two cordylid lizards, *Cordylus cataphractus* and *Cordylus polyzonus*. *Amphibia-Reptilia* **21**, 502-507.
- Mouton, P. le F. N., Geertsema, H. & Visagie, L. (2000c). Foraging mode of a group-living lizard, *Cordylus cataphractus* (Cordylidae). *African Zoology* **35**, 1-7.
- Myres, B. C. & Eells, M. M. (1968). Thermal aggregation in *Boa constrictor*. *Herpetologica* **24**, 61-66.
- Neill, W. T. (1948). Hibernation of amphibians and reptiles in Richmond County, Georgia. *Herpetologica* **4**, 107-114.
- Nieuwoudt, C. J., Mouton, P. le F. N., & Flemming, A. F. (2003a). Sex ratio, group composition and male spacing in the Large-scaled Girdled Lizard, *Cordylus macropholis*. *Journal of Herpetology* **37**, 577-580.
- Nieuwoudt, C. J., Mouton, P. le F. N., & Flemming, A. F. (2003b). Aggregating behaviour and movement patterns in the Large-scaled Girdled Lizard, *Cordylus macropholis*. *Amphibia-Reptilia* **24**, 345-357.
- Parzen, E. (1960). *Modern Probability Theory and its Applications*. New York: John Wiley & Sons, Inc.
- Peers, B. (1930). A record of the peculiarities of the lizard *Zonurus cataphractus* (Boie), as observed during travels in Namaqualand in May 1928. *South African Journal of Natural History* **VI**, 402-411.
- Pope, C. H. (1937). *Snakes alive and how they live*. New York: Viking Press.
- Rand, A. S. (1967). Communal egg-laying in anoline lizards. *Herpetologica* **23**, 227-230.
- Ruby, D. E. (1977). Winter activity in Yarrow's spiny lizard, *Sceloporus jarrovi*. *Herpetologica* **33**, 322-333.

- Seburn, C. N. L. (1993). Spatial distribution and microhabitat use in the five-lined skink (*Eumeces fasciatus*). *Canadian Journal of Zoology* **71**, 445-450.
- Stamps, J. A. (1988). Conspecific attraction and aggregation in territorial species. *American Naturalist* **131**, 329-347.
- Van Wyk, J. H. & Mouton, P. le F. N. (1992). Glandular epidermal structures of cordylid lizards. *Amphibia-Reptilia* **13**, 1-12.
- Visagie, L. (2001). Grouping behaviour in the armadillo lizard, *Cordylus cataphractus*. Unpublished M.Sc thesis, University of Stellenbosch, South Africa.
- Visagie, L., Mouton, P. le F. N. & Flemming, A. F. (2002). Intergroup-movement in a group-living lizard, *Cordylus cataphractus*, from South Africa. *African Journal of Herpetology* **51**, 75-80.
- Vitt, L. J. (1974). Winter aggregations, size classes, and relative tail breaks in the tree lizard, *Urosaurus ornatus ornatus* (Sauria: Iguanidae). *Herpetologica* **30**, 182-183.
- Weintraub, J. D. (1968). Winter behaviour of the granite spiny lizard, *Sceloporus orcutti* Stejneger. *Copeia* **1968**, 708-712.
- Worthington, R. D. & Sabath, M. D. (1966). Winter aggregations of the lizard *Urosaurus ornatus ornatus* (Baird and Girard) in Texas. *Herpetologica* **22**, 94-96.

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## FIELD BODY TEMPERATURES OF CAIMANS IN THE PANTANAL, BRAZIL

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Body temperatures of 51 caimans in the Pantanal were monitored by radio telemetry in cool (dry season) and warm (dry and wet seasons) seasons in an area with isolated lakes and an area with intermittent rivers. Cloacal temperatures of 739 caimans of different sizes captured between 1830 hrs and 2200 hrs were measured with a digital thermometer between August 1996 and September 1999 in the same area. The masses of caimans monitored ranged from 3 to 42 kg, and caiman size affected the amplitude of body temperatures. Small caimans generally had lower mean body temperatures than larger caimans only at the beginning of the night in the cool season before body temperatures equilibrated with water temperatures. Mean body temperature was 25.7 °C in the cool season and 30.1 °C in the warm season, with a minimum of 16.9 °C and a maximum of 37.9 °C. In the warm season, caimans spent more time in shady areas, on land or in the water, than exposed directly to sunlight, and body temperatures only slightly exceeded water temperatures. In the cool season, caimans basked in the sun, both on land and in water, and caimans on land achieved body temperatures up to 15 °C above water temperatures, but body temperatures of caimans on land rarely exceeded air temperatures during daylight hours. Gravid females did not have higher temperatures than females that were not gravid. The caimans appeared to vary from near thermoconformity in the warm season to active thermoregulation when water temperatures were less than about 28 °C. However, caimans often appeared to give low priority to thermoregulation, and much shuttling behavior may occur for reasons unrelated to thermoregulation.

*Key words:* behaviour, *Caiman crocodilus yacare*, temperature, thermoregulation, behaviour

### INTRODUCTION

Crocodylians are large semi-aquatic reptiles that thermoregulate actively by shuttling between air and water, and between places exposed to sun and places in the shade (Seebacher *et al.*, 1999). However, the significance of shuttling to the temperature relations of individuals may not be simple. *Alligator mississippiensis*, a temperate-zone species, basks to raise its temperature, but many species of crocodylians apparently leave the water to cool (Lang, 1987a; Thorbjarnarson, 1995). In *Crocodylus johnstoni*, some animals maintain temperatures similar to that of the water at all times of year, and others only achieve temperatures distinct from that of the water in some seasons (Seebacher & Grigg, 1997).

Although most species of crocodylians “bask” in the sun during the day (Lang, 1987a), it is not known whether this generally results in their attaining higher temperatures than they would have if they remained in water. As individuals may display shuttling behaviour as a result of social interactions (Lang, 1987b; Seebacher & Grigg, 1997), shuttling does not necessarily result in individuals attaining preferred body temperatures. Studies on turtles suggest that basking has other advantages, such as retarding growth of epiphytes and fungi on the skin (Moll & Legler, 1971), and may also be a strategy for the synthesis of vitamin D (Pritchard & Greenwood, 1968).

Tropical crocodylians avoid high ambient temperatures during much of the year, and Lang (1987a) considered them to be thermoconformers. However, detailed evaluation of the significance of behavioural changes often requires information on operative temperatures (Bakken & Gates, 1975; Tracy, 1982; O'Connor & Spotila, 1992; Hertz *et al.*, 1993), and studies on large numbers of wild individuals have been undertaken only on species of the genus *Crocodylus* (Grigg & Seebacher, 2001). Even in warm climates, seasonal changes in temperature can result in changes in shuttling behaviour and mean temperatures. Preferred temperatures also may change seasonally (Seebacher and Grigg, 1997). Habitat may also affect body temperatures in crocodylians (Loveridge, 1984), and female reptiles often seek higher temperatures when gravid (Shine, 1985).

Independent of the motivation for shuttling and whether the organism is actively thermoregulating, crocodylian size will affect body temperatures because large reptiles heat and cool slowly while smaller individuals warm and cool quickly (Spotila *et al.*, 1973; Smith, 1979; Grigg *et al.*, 1979; Stevenson, 1985; Seebacher, 1999; Seebacher *et al.*, 1999; Seebacher, 2000). During the day, small individuals could compensate for a lower thermal inertia by shuttling more frequently, or adopting heat-accumulating postures (Seebacher, 1999). However, crocodylians are aquatic, primarily-nocturnal predators. Heat loss is expected to be greatest at the beginning of the night when foraging

crocodilians return to the water, and opportunities to thermoregulate are limited. Due to their higher surface area to mass ratio, and the high specific heat of water, smaller crocodilians should lose heat much more quickly than large crocodilians when they return to the water at the beginning of the night and the water starts to cool (Grigg & Seebacher, 2001).

Although much information is available on the thermal relations of North American *Alligator mississippiensis* and Australian species of the genus *Crocodylus* (Grigg & Seebacher, 2001), little information is available on thermal relations in the wild for species from other regions. Shuttling behaviour has been described for *Caiman crocodilus crocodilus* in Venezuela (Staton and Dixon, 1975; Marcellini, 1979; Ayarzagüena, 1983; Thorbjarnarson 1995), but the body temperatures of the animals in different situations were not recorded.

In this paper we describe the behaviour and body temperatures of *Caiman crocodilus yacare* in the Brazilian Pantanal. *C. c. yacare* is one of the most abundant crocodilians (Coutinho & Campos, 1996; Mourão *et al.*, 2000), but there is no published information on its thermal relations. We used temperature-sensitive radio transmitters and direct measurements of cloacal temperature to investigate whether body temperature in *C. c. yacare* varies between seasons, between habitats, between animals of different reproductive states, and/or between animals of different sizes.

## MATERIALS AND METHODS

### STUDY AREA

The Pantanal is located in the upper Paraguay River basin, near the geographical centre of South America. The Pantanal covers almost 140,000 km<sup>2</sup>, and is characterized by a mosaic of seasonally flooded fields and slightly higher islands that do not flood. The climate is classified as AW (savanna) according to the Köppen system. The climate is markedly seasonal, with rain concentrated in late spring and summer (October to April). Winter and early spring (May to September) are generally warm and dry, except when cold fronts from the south cause abrupt falls in air temperature. The mean annual air temperature between 1986 and 1999 varied from 22 to 26 °C, and rainfall from 800 to 1200 mm/year (Soreano, 1997). Environmental temperatures during the study reflected the seasonally unpredictable temperatures in the Pantanal. From May to September, temperatures fluctuated widely. Hot days often alternated with cold days because of cold fronts from the south. Mean air temperatures were low in May 1998 (20.4 °C) and September 1996 (21.2 °C), but in September 1999 the mean air temperature was above 30 °C and the mean air temperature in April 1998 was 28 °C. In the remaining months of the year, mean air temperatures remained around 30 °C. The study was undertaken in the Nhecolândia region, located in the Taquari River alluvial fan in the south-west of the Pantanal.

*Major habitats and the monitoring of ambient temperatures.* One part of the study area (Campo Dora Ranch) is within the drainage basin of intermittent rivers and has few lakes, and the other part (Nhumirim Ranch) is in an area characterized by small shallow (<2.0 m) lakes. The river area is covered by open pastures and mosaics of gallery forest. The lake area is covered mainly by forest, but has some pastures. The lakes support a diverse aquatic plant community, but coverage, composition and biomass of macrophytes vary among lakes (Pott, 1994). During the rainy season, pastures are flooded, but during the dry season water remains only in larger lakes and in pools formed in the intermittent rivers.

Air and water temperatures were measured with StowAway<sup>Temp</sup> data-loggers (Onset®). They were programmed to take readings at the times that body temperatures were monitored. Water temperature was measured by the data-loggers near the surface (10-15 cm) and at 60 cm depth. Air temperature (TAR) was registered with data-loggers in shady places on the margins of lakes and rivers at 100 cm above ground level. In the river area, between October and November 1999, no data loggers were available, and air temperatures were registered in the Nhumirim Meteorological Station at 0800, 1400 and 2000 hrs. Operative temperatures were not measured. However, air and water temperatures probably indicate minimum temperatures available to caimans. We assumed that evaporative cooling would not be sufficient to maintain these large reptiles at temperatures much less than air temperature, and that evaporative cooling would not occur when the caimans were in water.

### RADIO TELEMETRY EQUIPMENT AND TEMPERATURE-SENSITIVE DATA LOGGERS

Caimans were located at night with spotlights, and approached from small boats in the pools in the river area, or on foot from the shore in the lake area, and captured with nooses attached to long poles. The caimans were taken to a laboratory at Nhumirim Ranch. The next morning, radio transmitters or data loggers were surgically implanted in the peritoneal cavity under sterile conditions.

During surgery, cooling was used for immobilization and 2 ml of Xylocaine for local anesthesia. Before initiating surgery, the incision area was washed with running water and neutral soap and sterilized with iodized alcohol. To cool the caimans, they were rolled in cloth, their legs tied, the mouth and eyes sealed with duct tape and they were placed in a freezer until their body temperatures dropped to about 19 °C (60-90 mins, depending on the size of the caiman). A surgical incision was made in the flank in front of the left hind leg. The radio transmitter was sterilized with iodized alcohol and introduced in the peritoneal cavity. The 47 cm antenna of the transmitter remained inside the body cavity. The peritoneal membrane and musculature were closed with absorbable sutures (catgut) in continuous points. The external inci-

sion was closed with cotton or nylon sutures, in individual points. All procedures followed ethical practices for animals recommended by EMBRAPA veterinarians. The animals did not show signs of stress and were released the same day at the site of capture.

The temperature-sensitive radio transmitters (Sirtrack®) measured approximately  $3 \times 2 \times 2$  cm, weighed 50 g, and had external 47 cm antennas. The data loggers (Onset® StowAway<sup>tdbit</sup>) measured 4 cm by 3 cm and weighed approximately 30 g. All radio transmitters and data loggers were encapsulated with resin at the factory. Radio transmitters had different frequencies in the 164 to 166 MHz range, and a predicted useful life of 6 months. The radios and data loggers were previously calibrated against a mercury bulb thermometer and readings were accurate to within 0.3 °C.

The radio signals were detected with a TR2-Scanner and a TR4 receiver (Telonics®). Radio transmitters could usually be detected at distances up to 500 m, especially when caimans were on banks or swimming at the surface of the water. A Sirtemp encoder® was used for body temperature reading.

The body temperatures of 739 caimans of different sizes captured between 1830 hr and 2200 hrs were measured with a digital thermometer between August 1996 and September 1999. Caimans were caught with a noose mounted on a pole and their cloacal temperatures were measured within 5 mins of capture.

#### DATES AND LOCATIONS OF MONITORING

The diel and seasonal variation in body temperatures of 51 caimans were monitored by temperature-sensitive radio transmitters and data loggers. In the lake area, one adult male was used to test the use of radio transmitters attached to the tail, implanted in the peritoneal cavity, and in the stomach in May 1995. We monitored body temperatures of one female from June to August 1997, 12 caimans (7 males and 5 females) from May to October 1998, 9 caimans (5 males and 4 females) from November to March 1998, and 5 caimans (2 males and 3 females) from May to August 1999. In the river area, we monitored body temperatures of 10 caimans (8 males and 2 females) from June to July 1999, and 14 caimans (7 males and 7 females) from October to November 1999.

In the lake area, monitoring was done over 3-4 consecutive days, at intervals of one hour in the cool season (May - September) and in the warm season (October - March). In the intermittent-river area, monitoring was done during 7 to 9 consecutive days, at intervals of 1 hour in the cool season (June - July) and in the warm season (October - November).

#### REPRODUCTIVE STATE

No attempt was made to determine the reproductive state of males, but five of the females had pre-ovulatory follicles in advanced stages of development as evidenced by the flaccid cloaca, swollen flanks and increased body weight.

#### OBSERVATIONS OF CAIMAN BEHAVIOUR

Free-ranging caimans with radio transmitters were marked individually with coloured collars to facilitate observations of basking behaviour. At the same time that body temperatures of caimans were recorded, observations were made of basking behaviour of the caimans with radio transmitters. Each hour, the positions of the caimans (on land or in water, and in sun or in shade) were recorded. In the river area, which had open margins without vegetation, caimans with radio transmitters could be observed when in water or on land. In the lake area, basking caimans could not be observed due to aquatic vegetation. In this area, caimans were rarely found on the margins of the lakes, but rested on floating vegetation when out of the water. All analyses were done in the Systat version 8.0 program (Wilkinson, 1998).

## RESULTS

#### SEASONAL VARIATION IN BODY TEMPERATURE

In this study, we registered 17661 body temperatures by radio telemetry and data loggers from 51 individuals over three years (Table 1). The minimum temperature registered was 16.9 °C and the maximum 37.4 °C. The mean water and air temperatures were more strongly correlated in the warm ( $r=0.96$ ) season than in the cool ( $r=0.80$ ) season when cold fronts from the south often resulted in short-term abrupt changes in air temperature that were reflected only weakly by water temperatures. The only cold front that passed through the study site during a monitoring period occurred in September, and resulted in caiman body temperatures well below those recorded in other months in the warm or cool seasons (Fig. 1).

The mean body temperature in the cool season was 25.7°C (Median=25.7, SD=0.86), and the mean monthly body temperature varied from 23.8 to 28.3 °C. In the warm season, mean body temperature was 30.1 °C (Median=30.3, SD=0.66), and mean monthly temperatures varied from 28.5 to 31.3 °C. Mean monthly body temperatures were significantly ( $t_{49}=20.6$ ,  $P<0.001$ ) lower in the cool season than in the warm season (Fig. 1). This difference persisted ( $t_{39}=17.3$ ,  $P<0.001$ ) when we excluded September, the month in which data recording coincided with the passage of a cold front.

Maximum body temperatures registered for each individual were also significantly ( $t_{49}=2.57$ ,  $P=0.013$ ) lower in the cool season (mean=34.2°C, median=34.2, min=31.5, max=37.2) than in the warm season (mean=35.1°C, median=35.4, min=31.5, max=37.4), but there was no significant difference ( $t_{39}=1.49$ ,  $P=0.145$ ) between maximum temperatures in the cool season (mean<sub>max</sub>=34.4°C, median=34.3, min=31.5, max=37.2) and the warm season (mean<sub>max</sub>=35.1°C, median=35.4, min=31.5, max=37.4) when we excluded September, the month in which data recording coincided with the passage of a cold front.

TABLE 1. Snout-vent lengths (SVL), and body temperatures (BT - °C) of caimans in the lake (L) and river (R) areas in cool (C) and warm (H) seasons.

Sex	SVL (cm)	Mass (kg)	Season	Year	$O_{TB} \pm SD$	Minimum	Maximum	N	Habitat
F	61.0	4.8	C	1999	26.0 ± 3.3	20.5	34.3	315	L
F	62.5	5.1	C	1998	26.2 ± 4.1	17.3	33.8	490	L
F	63.0	5.2	C	1998	28.3 ± 4.1	17.3	35.6	443	L
F	68.0	6.4	H	1998	30.9 ± 2.1	26.8	36.2	221	L
F	69.0	7.5	H	1998	28.5 ± 3.6	24.9	33.5	610	L
F	70.0	7.0	C	1997	25.2 ± 3.0	19.8	34.3	85	L
F	72.0	7.6	H	1998	31.3 ± 2.1	25.9	36.9	224	L
F	72.0	7.4	H	1998	30.9 ± 1.9	26.4	36.0	231	L
F	73.5	8.0	C	1999	25.7 ± 3.0	21.3	34.6	295	L
F	77.0	10.0	C	1999	23.8 ± 2.6	20.1	31.5	91	L
F	77.0	11.5	C	1998	26.1 ± 3.4	17.6	33.5	486	L
F	78.0	12.0	C	1998	26.1 ± 3.4	17.6	33.6	490	L
F	83.0	13.0	C	1998	30.7 ± 1.6	26.5	35.9	240	L
M	55.0	3.3	C	1999	20.7 ± 3.1	20.7	33.6	302	L
M	60.0	4.3	C	1998	25.5 ± 3.3	17.6	32.9	490	L
M	63.0	4.7	C	1998	26.2 ± 3.8	17.0	34.4	487	L
M	64.0	5.5	C	1998	25.5 ± 2.1	21.0	31.9	256	L
M	75.0	7.8	H	1998	30.5 ± 1.5	26.3	34.4	241	L
M	82.0	11.0	C	1999	25.2 ± 2.9	20.2	34.0	281	L
M	83.0	12.0	H	1998	30.6 ± 1.7	26.5	35.5	249	L
M	85.0	13.0	C	1998	26.1 ± 3.5	17.8	32.9	465	L
M	86.0	15.0	C	1998	25.7 ± 3.6	17.2	33.4	489	L
M	94.5	19.5	H	1998	30.5 ± 1.5	27.1	34.7	223	L
M	95.5	20.0	C	1998	25.9 ± 3.2	17.8	32.8	490	L
M	106.0	27.0	H	1998	30.6 ± 1.4	26.6	33.8	207	L
M	106.0	25.0	H	1998	29.7 ± 3.7	17.9	37.4	4420	L
M	113.0	31.5	C	1998	25.5 ± 3.3	17.6	32.9	482	L
F	60.0	5.0	H	1999	30.2 ± 3.2	22.9	36.3	212	R
F	69.0	8.0	C	1999	28.3 ± 4.1	20.5	34.2	223	R
F	72.0	9.5	H	1999	29.8 ± 3.1	22.2	35.9	228	R
F	74.0	8.0	C	1999	24.7 ± 3.9	16.9	35.8	289	R
F	77.5	13.0	H	1999	28.8 ± 3.1	23.9	33.3	31	R
F	81.0	14.0	H	1999	30.9 ± 2.3	26.5	35.7	139	R
F	84.0	15.0	H	1999	30.3 ± 2.6	24.8	34.7	114	R
F	89.0	19.0	H	1999	29.9 ± 2.6	24.2	34.6	198	R
F	90.0	17.0	H	1999	29.2 ± 2.3	24.2	34.7	133	R
M	57.0	3.9	C	1999	25.0 ± 3.9	19.4	35.4	176	R
M	58.0	4.0	C	1999	25.2 ± 4.8	17.4	37.2	139	R
M	63.5	6.0	C	1999	25.4 ± 4.1	17.4	35.1	272	R
M	75.0	10.0	H	1999	30.3 ± 2.7	23.4	36.2	167	R
M	79.0	10.0	C	1999	27.3 ± 5.4	19.2	35.1	16	R
M	80.5	13.0	H	1999	29.8 ± 2.1	24.0	35.1	209	R
M	83.5	15.0	H	1999	29.9 ± 3.1	23.6	35.7	162	R
M	88.5	16.5	H	1999	29.8 ± 2.8	23.0	35.1	227	R
M	89.0	12.0	C	1999	25.2 ± 3.6	20.0	36.1	188	R
M	99.0	22.0	C	1999	24.9 ± 3.7	17.4	33.7	326	R
M	101.0	22.5	H	1999	30.2 ± 2.9	23.6	35.4	166	R
M	105.0	29.0	H	1999	29.7 ± 2.8	23.5	35.3	218	R
M	111.0	28.0	C	1999	24.5 ± 3.9	17.6	34.2	301	R
M	115.0	39.0	H	1999	29.7 ± 2.7	24.1	35.8	213	R
M	122.0	42.0	C	1999	30.4 ± 1.2	27.5	31.5	11	R

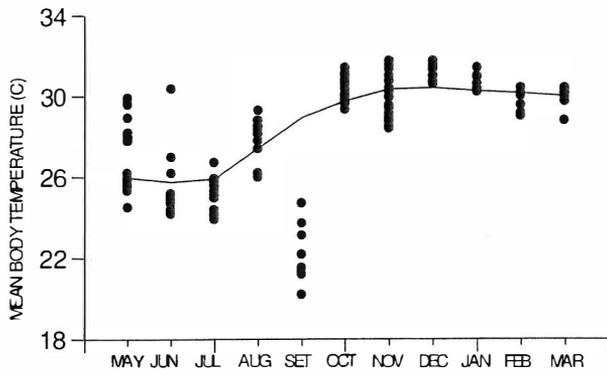


FIG. 1. Mean monthly body temperatures of individual caimans. The curve is a LOWESS regression (tension=0.5).

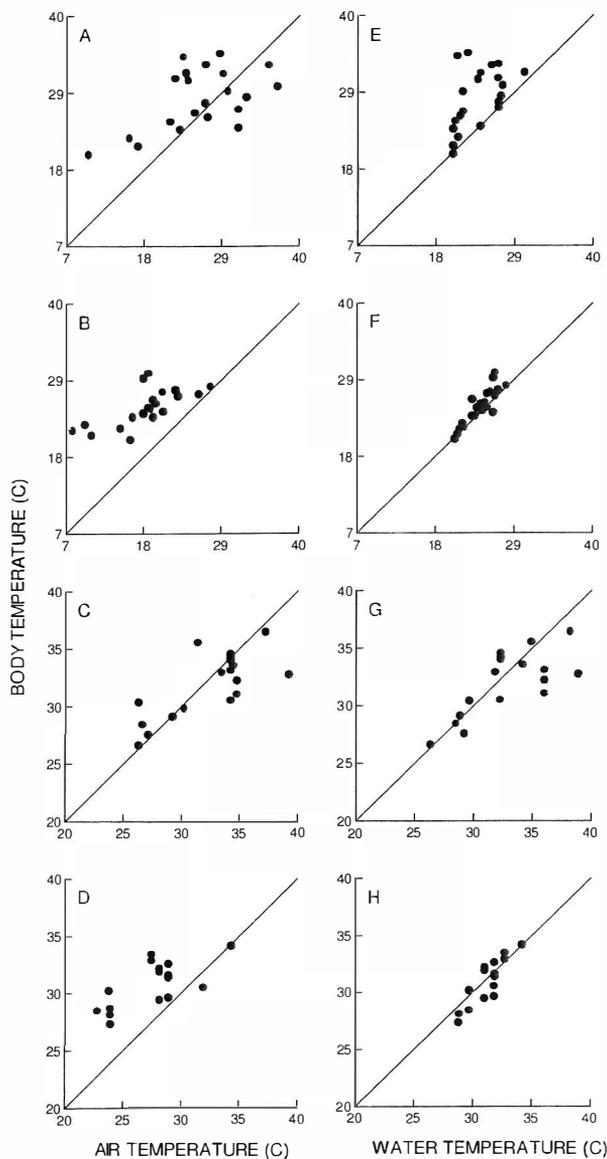


FIG. 2. Relationship between body temperature and air temperatures during the day (A) and during the night (B) in the cool season, and during the day (C) and during the night (D) in the warm season. Relationship between body temperature and surface water temperature during the day (E) and during the night (F) in the cool season, and during the day (G) and during the night (H) in the warm season.

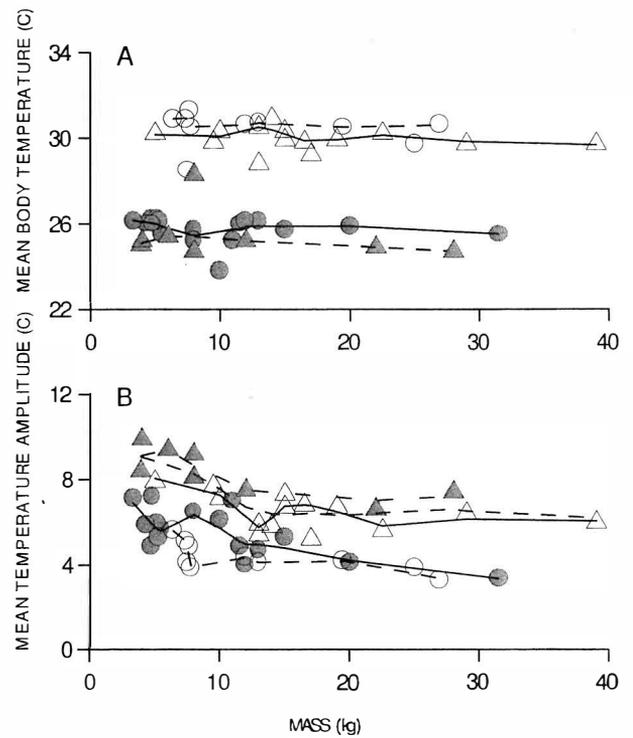


FIG. 3. Relationship between mass and body temperature ( $^{\circ}\text{C}$ ) (A), and mean daily amplitude in temperature ( $^{\circ}\text{C}$ ) (B) for caimans in cool (filled circles) and warm (open circles) seasons in the lake area, and cool (filled triangles) and warm (open triangles) seasons in the river area.

MEAN DIURNAL AND NOCTURNAL TEMPERATURES

Hourly records of body temperatures obtained by radio telemetry, coupled with hourly records of air and water temperatures, were available for 21 caimans in the cool season and 18 caimans in the warm season. Mean body temperature was correlated with mean air ( $r=0.51$ ) and mean water ( $r=0.55$ ) temperature during the day, and mean air ( $r=0.88$ ) and mean water ( $r=0.70$ ) temperature during the night in the cool season; and mean air ( $r=0.74$ ) and mean water ( $r=0.76$ ) temperature during the day, and mean air ( $r=0.85$ ) and mean water ( $r=0.70$ ) temperature at night in the warm season (Fig. 2). Mean temperatures higher than those of the water were obtained mainly during the day in the cool season. The mean body temperature at night varied from 24.9 to 33  $^{\circ}\text{C}$ . Over the whole study period, mean monthly temperature of caimans at night was strongly correlated with mean monthly air ( $r=0.83$ ,  $N=10$ ) and water ( $r=0.97$ ,  $N=10$ ) temperatures.

EFFECTS OF BODY MASS AND HABITAT ON BODY TEMPERATURE

The body mass of the caimans monitored by radio telemetry ranged from 3.3 to 42 kg (Table 1) and was log transformed for statistical analyses. Analysis of covariance (ANCOVA) included site (lake or river) and season (cool or warm), as categorical variables. Mean body temperature (Fig. 3A) was affected by season ( $F_{1,47}=378.6$ ,  $P<0.001$ ) but not site ( $F_{1,47}=1.01$ ,  $P=0.319$ ) or  $\text{Log}_{10}\text{Mass}$  ( $F_{1,47}=1.15$ ,  $P=0.289$ ). The

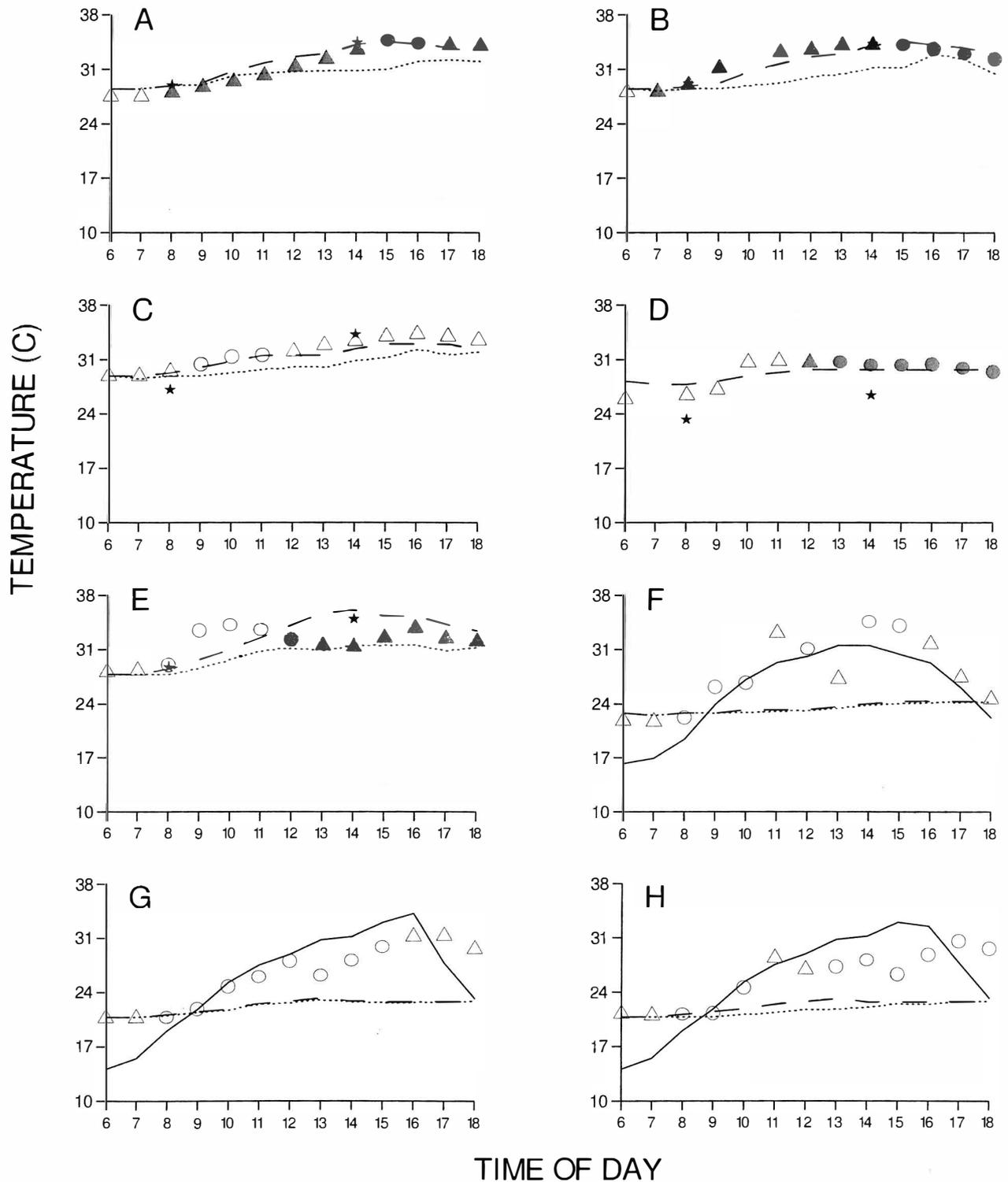


FIG. 4. Relationship between body temperature (circles and triangles), air temperature (asterisks or solid line), water temperature at 60 cm depth (dotted line), surface water temperature (dashed line), and time of day. Triangles indicate that the individual was in the water and circles indicate that it was on land. Open symbols indicate that the individual was exposed to the sun, and closed symbols indicate that it was in the shade.

mean daily amplitude (Fig. 3B) of body temperature was affected significantly by season ( $F_{1,47}=12.6, P=0.001$ ), site ( $F_{1,47}=59.4, P<0.001$ ) and  $\text{Log}_{10}\text{Mass}$  ( $F_{1,47}=0.14.0, P<0.001$ ).

DIURNAL PATTERNS IN BODY TEMPERATURES

In the river area, eight caimans were observed between 0600 and 1800 hrs. In the warm season, five

caimans (two females and three males) had similar patterns of terrestrial-aquatic movements and body temperatures, which were close to surface water temperatures (Fig. 4A, B, C, E, F). Body temperatures during the warm season were around 28 °C in the early morning and usually approached 35 °C at midday. The two females remained in water or on land in the shade (Fig. 4A, B). However, the males (Fig. 4C, D, E) re-

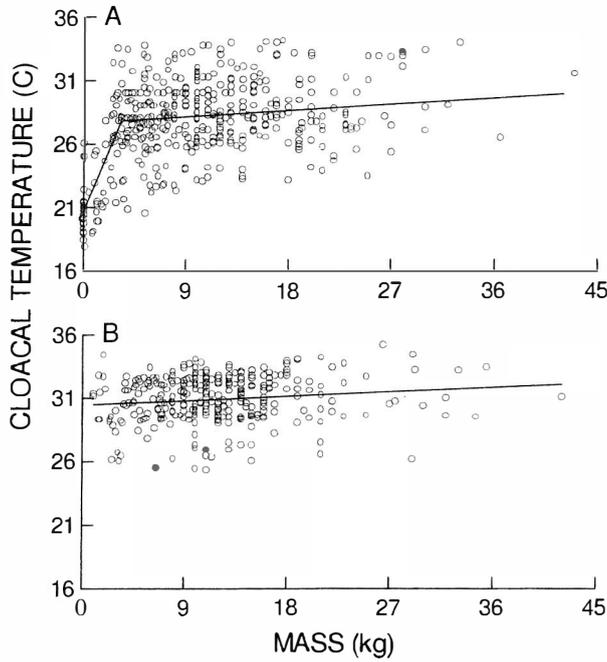


FIG. 5. Relationship between cloacal temperature and mass of caimans in the early hours of the night (1830-2200 hrs) during the cool (A) and warm (B) seasons.

mained in the water exposed to sun in the early morning, and alternated between land and water after 1200 hrs. During the warm season, body temperatures of caimans were similar to surface water temperatures, independent of whether they were in the shade or exposed to sun, or whether they were on land or in the water.

In the cool season, the body temperatures of the three caimans were closer to air temperature than water temperature during daylight hours, and the animals spent more time exposed to the sun, on land and in water (Fig. 4 F, G, H). The mean body temperature in the morning in the cool season was around 23 °C, rising to over 30 °C, for a few hours at midday.

NOCTURNAL CLOACAL TEMPERATURES

The body temperatures of 739 caimans of different sizes were measured at night (1830-2200 hrs) between August 1996 and September 1999. The snout-vent lengths of the caimans captured ranged from 14.5 to 120 cm (mean=74.0, SD=20.4) and mass varied from 0.04 to 43.0 kg (mean=10.7, SD=6.9). During the cool season (May to September), there was a nonlinear relationship between mean cloacal temperature (CT) and body mass (Fig. 5A). Piecewise regression indi-

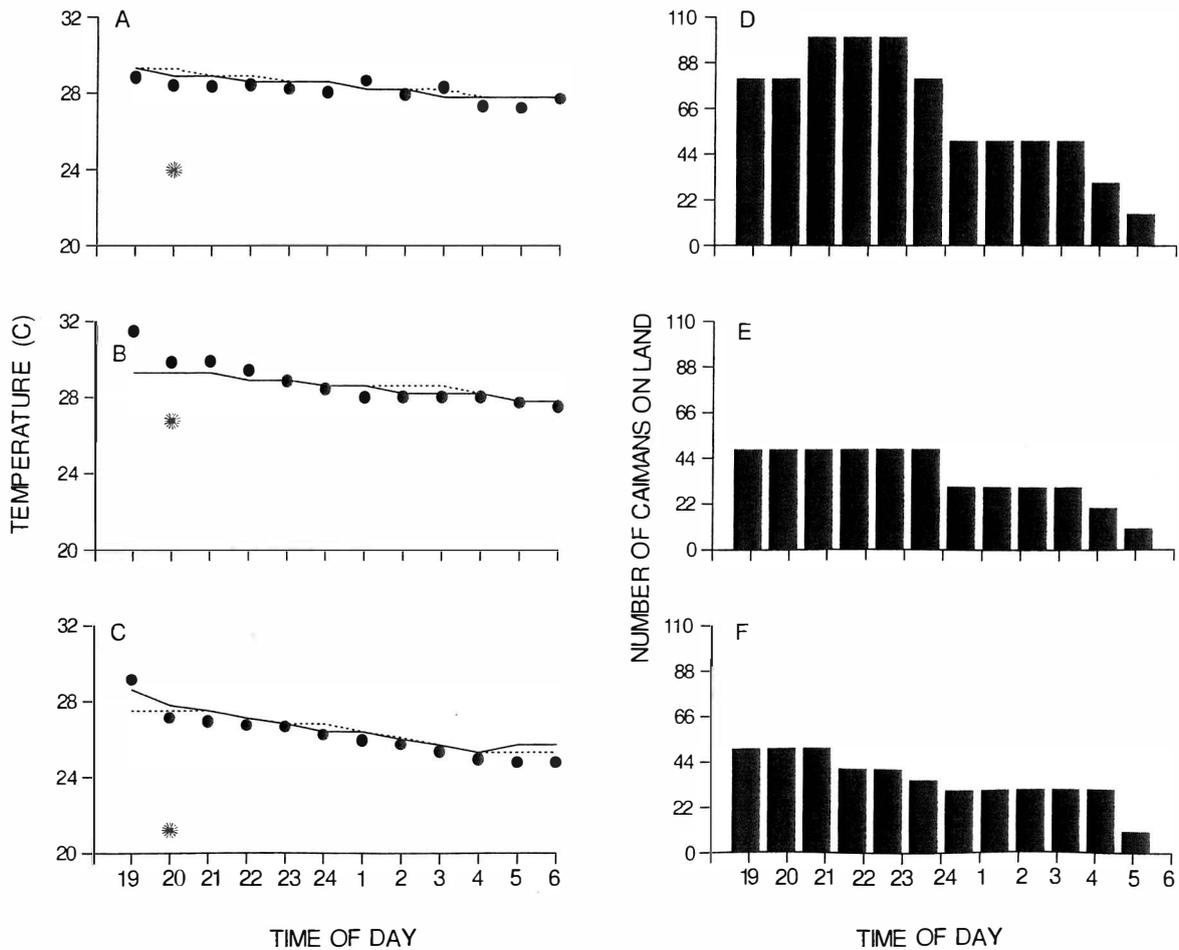


FIG. 6. Mean body temperatures of 14 caimans in a pool in the river (filled circles), air temperature (asterisks), water temperature at the surface (solid line), and water temperature at 60 cm depth (dotted line) between 1900 hrs and 0600 hrs on 28 October (A), 29 October (B), and 6 November 1999. Numbers of caimans on land beside the pool at hourly intervals on 28 October (D), 29 October (E), and 6 November 1999 (F).

cated a very strong relationship between mass and temperature for caimans with masses of <3.3 kg ( $CT=20.7+2.167 \times \text{mass}$ ,  $r^2=0.494$ ,  $N=73$ ,  $P<0.001$ ), and a much smaller effect of mass on mean nocturnal temperature for larger animals ( $CT=27.6+0.055 \times \text{mass}$ ,  $r^2=0.020$ ;  $N=358$ ,  $P=0.008$ ).

In the warm season (October to April), there were no hatchlings in the population and individuals with body masses <3.3 kg had temperatures similar to those of adults (Fig. 5B). In this season, body mass explained only about 1.5% of the variation in nocturnal body temperatures ( $CT=30.6+0.035 \times \text{mass}$ ,  $r^2=0.015$ ,  $N=316$ ,  $P=0.035$ ).

#### SHUTTTLING BEHAVIOUR AT NIGHT

Caimans in a pool in the river area were observed at night on 28-29 October, and 6 November 1999. The number of caimans on land was estimated at hourly intervals. As the caimans had to be illuminated with a spotlight during counts, the number of caimans was estimated quickly in intervals of 10 to avoid disturbance. The caimans undertook movements between water and land similar to those undertaken during the day, and there were large numbers of caimans on land throughout the night, until about 0500 hrs (Fig. 6D, E, F). Fewer caimans were seen on land on the second and third nights, but it is not known whether this resulted from a reduction in number of caimans using the pool, or a reduction in the proportion leaving the water.

The body temperatures of 14 caimans in the pool were monitored by radio telemetry during the observation period. We could not distinguish individual caimans at night, but assume that the monitored caimans showed similar shuttling behaviour to the other individuals. Air temperatures were well below water temperatures at the beginning of the night, and the difference probably increased throughout the night. The mean temperature of the 14 caimans was similar to water temperatures, and decreased slowly over the night (Fig. 6A, B, C).

#### TEMPERATURES OF GRAVID FEMALES

Five females with well developed ovarian follicles were monitored in the warm season. Mean body temperatures ( $t_{4.3}=-0.412$ ,  $P=0.70$ ) and standard deviations of body temperature ( $t_{7.2}=-1.533$ ,  $P=0.168$ ) of those females were similar to those of other females.

#### DISCUSSION

Body temperatures of free ranging *Caiman crocodilus yacare* varied strongly throughout the year, in concert with ambient temperatures, and the range of temperatures recorded (16.9-37.4 °C) was similar to those recorded in the literature for captive and free ranging crocodilians (Colbert *et al.*, 1946; Cott, 1961; Johnson *et al.*, 1976; Diefenbach, 1975; Grigg *et al.*, 1998). In the cool months, with alternating cold and hot days, the mean body temperature of caimans (26 °C) was lower than in the hotter part of the year (30 °C). Pre-

ferred temperatures may change seasonally in *Crocodylus johnstoni* (Seebacher & Grigg, 1997). We did not record operative temperatures, and therefore cannot address preferred temperatures in the Pantanal caiman. However, excluding one period in which a strong cold front passed through the study site, maximum temperatures did not vary between the warm and cool seasons, suggesting that the caimans were attempting to attain similar temperatures in both seasons.

Several authors have discussed the effect of body mass on temperature patterns shown by crocodilians (Smith, 1979; Diefenbach, 1975), but only Grigg *et al.* (1998) demonstrated an effect, and then only for very large (>100 kg) individuals. Variation in mass of caimans (3 to 42 kg) was not significantly related to the mean temperature of caimans, but significantly affected the amplitude of temperature fluctuations. Temperatures of small caimans fluctuated more than those of large caimans. The smaller fluctuation of body temperatures of large individuals has been attributed to thermal inertia in large lizards, turtles and crocodilians (Read *et al.*, 1996; Grigg *et al.*, 1998).

Although mass generally had a negligible effect on mean body temperatures of caimans, it was important in specific periods. At the beginning of the night, body temperatures of small caimans were lower than large caimans, especially in the cool season, probably due to the high specific heat of water, and the larger surface area to mass ratio of small caimans (Grigg & Seebacher 2001). As caimans forage principally at night (Lang, 1987a), differences in body temperature could have a disproportionate effect on fitness during this period.

In the river area, the amplitude of daily temperature fluctuations was greater than in the lake area. Habitat affects survival of eggs and sex ratios of hatchlings (Campos, 1993), but little is known of the effect of habitat on survival and growth of caimans post hatching. As the lakes have more cover, lower densities of large caimans, and lower temperature fluctuations, they may be important resources for small caimans, especially in the dry season.

Water has a fundamental role in thermoregulation of crocodilians (Lang, 1987b), because thermal conductance and specific heat of water are higher than those of the air. Caimans left the water during the day and the night during both seasons. However, they obtained temperatures well above those of the water only during the day in the cool season. During the warm season, body temperatures were similar to water temperatures, independent of whether the caimans were on land or in the water, and whether they were in the sun or in the shade. As the effect of shuttling onto land on body temperatures was minimal during this season, much of the shuttling may have taken place for reasons other than thermoregulation. Caimans possibly moved between the water and land for social reasons (Lang, 1987a; Seebacher & Grigg, 1997) and/or to reduce the growth of fungi, algae or bacteria on the skin (Moll & Legler, 1971; Pritchard & Greenwood, 1968). In the months of

October and November, the lakes and pools in the rivers become very shallow and algae and bacteria accumulate due to the high density of caimans and dying fish (Campos *et al.*, 1994).

When brooding eggs, *Python molurus* can regulate its body temperature, and the temperature of its eggs, by increasing metabolic rate through muscular contractions (Van Mierop & Barnard, 1976). Female lizards may decrease their body temperatures when gravid (Tosini & Avery, 1996). However, in this study, the body temperatures of five female caimans in the river area with large ovarian follicles were similar to those of female caimans without well developed ovarian follicles. Gravid female lizards may seek higher temperatures because the eggs are kept in the oviducts for long periods after fertilization and females can control the temperature of embryos (Yaron, 1985). Embryos of crocodylians are less advanced at the time of laying than those of most lizards and snakes (Hubert, 1985), with most development occurring in the nest (Ferguson, 1987). It is the nest that keeps the temperatures of eggs relatively high and constant (Magnusson *et al.*, 1990).

The caimans seemed to be largely thermoconformers in the warm season when air and water temperatures generally were above 28 °C. In the cool season, when water temperatures were around 20 °C, the caimans alternated between the water and the air depending on which was hotter. It is probable that the majority of the crocodylians are usually thermoconformers, in the sense that thermoregulation has a lower priority than activities such as hunting, social interactions, and the need to avoid parasites in the water, when available water temperatures are above 28 °C. However, the caimans seemed to thermoregulate actively, selecting warmer positions when water temperatures were below 28 °C. It is probable that the capacity to accept relatively low temperatures is necessary for nocturnal aquatic predators that have few options to thermoregulate during their period of greatest foraging activity.

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#### REFERENCES

- Ayarzagüena, J. S. (1983). Ecología del caiman de anteojos o baba (*Caiman crocodilus* L.) en los llanos de Apure (Venezuela). *Doñana Acta Vertebrata* **10**, 7-136.
- Bakken, G. S. & Gates, D. M. (1975). Heat transfer analysis in animals: some implications for field ecology, physiology and evolution. In *Perspectives of Biophysical Ecology*, 255-290. Gates, D. M. & Schmerl, R. B. (Eds). New York, Springer Verlag.
- Campos, Z. (1993). Effect of habitat on survival of eggs and sex ratio of hatchlings of *Caiman crocodilus* *yacare* in the Pantanal, Brazil. *Journal of Herpetology* **27**, 127-132.
- Campos, Z., Coutinho, M. & Abercrombie, C. (1994). Night-light counts, size structure, and sex ratio in wild populations of *yacare* caiman (*Caiman crocodilus yacare*) in the Brazilian Pantanal. *Vida Silvestre Neotropical* **4**, 46-50.
- Colbert, E. H., Cowles, R. B. & Bogert, C. M. (1946). Temperature tolerances in the American alligator and their bearing on the habitats, evolution, and extinction of the dinosaurs. *Bulletin American Museum of Natural History* **86**, 327-374.
- Cott, H. B. (1961). Scientific results of an inquiry into the ecology and economic status of the Nile crocodile (*Crocodylus niloticus*) in Uganda and northern Rhodesia. *Transactions of the Zoological Society London*. **29**, 211-356.
- Coutinho, M. & Campos, Z. (1996). Effect of habitat and seasonality on the densities of caiman in southern Pantanal, Brazil. *Journal of Tropical Ecology* **12**, 741-747.
- Diefenbach, C. O. (1975). Thermal preference and thermoregulation in *Caiman crocodilus*. *Copeia* **1975**, 530-40.
- Ferguson, M. W. J. (1987). Post-laying stages of embryonic development for crocodylians. In *Wildlife management: Crocodiles and Alligators*, 409-415. Webb, G. W., Manolis, S. C. & Whitehead, P. J. (Eds). Chipping Norton, Surrey Beatty & Sons.
- Grigg, G. C. & Seebacher, F. (2001). Crocodylian thermal relations. In *Crocodylian Biology and Evolution*, 297-309. Grigg, G. C., Seebacher, F. & Franklin, C. E. (Eds). Chipping Norton, Surrey Beatty and Sons.
- Grigg, G. C., Drane, C. R. & Courtice, G. P. (1979). Time constants of heating and cooling in the eastern water dragon, *Physignathus lesseuri* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology* **4**, 95-103.
- Grigg, G. C., Seebacher, F., Beard, L. & Morris, D. (1998). Thermal relations of large crocodiles, free-ranging in a naturalistic situation. *Proceedings of the Royal Society London* **265**, 1793-1799.
- Hertz, P. E., Huey, R. B. & Stevenson, R. D. (1993). Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist* **142**, 796-818.
- Hubert, J. (1985). Embryology of marine turtles. In *Biology of the Reptilia, Volume 15*, 269-328. Gans, C. & Pough, F. H. (Eds). New York, John Wiley and Sons.
- Johnson, C. R., Webb, G. J. W. & Tanner, C. (1976). Thermoregulation in crocodylians - II. A telemetric study of body temperature in the Australian crocodiles, *Crocodylus johnstoni* and *Crocodylus porosus*. *Comparative Biochemistry and Physiology* **53A**, 143-146.
- Lang, J. W. (1987a). Crocodylian thermal selection. In *Wildlife Management: Crocodiles and Alligators*, 301-317. Webb, G. J., Manolis, C. & Whitehead, P. J. (Eds). Chipping Norton, Surrey Beatty and Sons.

- Lang, J. W. (1987b). Crocodylian behaviour: implications for management. In *Wildlife Management: Crocodiles and Alligators*, 273-294. Webb, G. J., Manolis, C. & Whitehead, P. J. (Eds). Chipping Norton, Surrey Beatty and Sons.
- Loveridge, J. P. (1984). Thermoregulation in the Nile crocodile, *Crocodylus niloticus*. In *The Structure, Development and Evolution of Reptiles*, 443-468. Ferguson, M. W. J. (Ed.). London, Academic Press.
- Magnusson, W. E., Lima, A. P., Hero, J.-M., Sanaiotti, T. M. & Yamakoshi, M. (1990). *Paleosuchus trigonatus* nests: sources of heat and embryo sex ratios. *Journal of Herpetology* **24**, 397-400.
- Marcellini, D. L. (1979). Activity patterns and densities of Venezuelan caiman (*Caiman crocodilus*) and pond turtles (*Podocnemis vogli*). In *Vertebrate Ecology in the Neotropics*, 263-271. Eisenberg, J. (Ed.). Washington, Smithsonian Institution Press.
- Moll, E. O. & Legler, M. (1971). The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff), in Panama. *Bulletin Los Angeles Museum of Natural History* **11**, 1-102.
- Mourão, G., Coutinho, M., Mauro, R., Campos, Z., Tomás, W. & Magnusson, W. (2000). Aerial surveys of caiman, marsh deer and pampas deer in the Pantanal wetland of Brazil. *Biological Conservation* **92**, 175-183.
- O'Connor, M. P. & J. R. Spotila. (1992). Consider a spherical animal: when do short cuts matter in biophysical models? *American Zoologist* **32**, 179-193.
- Pott, A. (1994). Ecosistema Pantanal. In *Utilización y Manejo de Pastizales*, 31-44. Montevideo, IICA/ PROCISUR Diálogo 40.
- Pritchard, P. C. H. & Greenwood, W. F. (1968). The sun and the turtle. *International Turtle and Tortoise Society Journal* **2**, 20-25.
- Read, M. A., Grigg, G. C. & Limpus, C. J. (1996). Body temperatures and winter feeding in immature green turtles, *Chelonia mydas*, in Moreton Bay, Southeastern Queensland. *Journal of Herpetology* **30**, 262-265.
- Seebacher, F. (1999). Behavioral postures and the rate of body temperature change in wild freshwater crocodiles, *Crocodylus johnstoni*. *Physiological and Biochemical Zoology* **72**, 57-63.
- Seebacher, F. (2000). Heat transfer in a microvascular network: the effect of heart rate on heating and cooling in reptiles (*Pogona barbata* and *Varanus varius*). *Journal of Theoretical Biology* **203**, 97-109.
- Seebacher, F. & Grigg, G. C. (1997). Patterns of body temperature in wild freshwater crocodiles, *Crocodylus johnstoni*: thermoregulation vs. conformity, seasonal acclimatisation and the effect of social interactions. *Copeia* **1997**, 549-557.
- Seebacher, F., Grigg, G. C. & Beard, L. A. (1999). Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *Journal of Experimental Biology* **202**, 77-86.
- Shine, R. (1985). The evolution of viviparity in reptiles: an ecological analysis. In *Biology of the Reptilia, Volume 15*, 605-694. Gans, C and Billet, F. (Eds). New York: John Wiley and Sons.
- Smith, E. N. (1979). Behavioral and physiological thermoregulation of crocodylians. *American Zoologist* **19**, 239-247.
- Soreano, B. A. (1997). *Boletim Agrometeorológico: 1989-1996*. Corumbá, Empresa Brasileira de Pesquisa Agropecuária - CPA Pantanal.
- Spotila, J. R., Lommen, P. W., Bakken, G. S. & Gates, D. M. (1973). A mathematical model for body temperatures of large reptiles: implications for dinosaur ecology. *American Naturalist* **107**, 391-404.
- Staton, M. A. & Dixon, J. R. (1975). Studies on the dry season biology of *Caiman crocodilus crocodilus* from the Venezuelan Llanos. *Memorias Sociedad Ciencias Naturales* **35**, 237-266.
- Stevenson, R. D. (1985). Body size and the limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist* **125**, 102-117.
- Thorbjarnarson, J. B. (1995). Dry season diel activity patterns in spectacled caiman (*Caiman crocodilus*) in the Venezuelan llanos. *Amphibia-Reptilia* **16**, 415-421.
- Tosini, G. & Avery, R. (1996). Pregnancy decreases set point temperatures for behavioural thermoregulation in the wall lizard *Podarcis muralis*. *Herpetological Journal* **6**, 94-96.
- Tracy, C. R. (1982). Biophysical modelling in reptilian physiology and ecology. In *Biology of the Reptilia, Volume 12*, 275-321. Gans, C. & Pough, F. H. (Eds). New York, Academic Press.
- Van Mierop, L. H. S. & Barnard, S. M. (1976). Thermoregulation in a brooding female *Python molurus bivittatus* (Serpentes: Boidae). *Copeia* **1976**, 398-401.
- Wilkinson, L. (1998). SYSTAT: The System for Statistics. Evanston, SYSTAT Inc.
- Yaron, Z. (1985). Reptilian placentation and gestation: structure, function, and endocrine control. In *Biology of the Reptilia*, 528-603. Gans, C. & Billet, F. (Eds.). New York, John Wiley and Sons.

## SOIL ACIDIFICATION NEGATIVELY AFFECTS EMBRYONIC DEVELOPMENT OF FLEXIBLE-SHELLED LIZARD EGGS

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Many reptile species dig underground nests where they deposit eggs with flexible and permeable shells that have physical contact with the soil and are highly permeable to soil water and gases. Iberian rock lizard eggs (*Lacerta monticola cyrenni*) incubated in acidic substrates suffered significant impairment to their development. Therefore, soil pollution could be affecting embryonic development. Low pH had a negative effect on egg water exchange, hatchling size and locomotor performance. In all cases, pH had no effect on incubation duration and embryo survival. At substrate pH of 4 and 10, eggs absorbed less water and final egg size and weight was lower than at neutral pH. Hatchlings from eggs incubated in acidic substrates had lower mass (up to 28 % of weight), SVL, and tail length than controls. Running speed – a good indicator of lizard fitness – was also affected by substrate pH. Embryos incubated at pH 4 ran slower than controls. The alteration of the water absorption process that low substrate pH had on eggs during incubation partially explained the observed effects on hatchling characteristics. These sublethal effects may influence the survival or success of juveniles during or after their first wintering.

*Key words:* ecotoxicology, incubation, lacertids, soil pH

### INTRODUCTION

Soil pollution, and more specifically the alteration of soil pH, may influence embryonic development and hatching success of these species. They usually inhabit areas that are susceptible to acidification such as industrialized areas, conifer forests, heathlands, moorlands or mountain grasslands. This impact could be particularly significant on species that have flexible and permeable shelled eggs, such as terrestrial egg-layer amphibians and many reptiles (Packard & Packard, 1988). During incubation, flexible-shelled eggs exchange gases and water with the nest environment. Eggs can absorb water from the soil increasing their weight and volume up to three or four times. Water absorption and gas exchange are necessary to successfully complete embryonic development (Thompson, 1987; Overall, 1994).

We were interested in the study of the impact of soil quality on flexible-shelled reptile eggs and, more specifically, the effect of soil pH on reptile embryonic development. Extreme soil pHs could have detrimental effects on the eggshell or egg content. Soil pH could also modify the egg environment, thus influencing water or gas exchanges between the egg and the surrounding soil, or it could directly modify egg content pH, and therefore affect embryonic development. To test the sensitivity of flexible shelled reptile eggs to soil pH, we incubated Iberian rock lizard eggs from mountain areas in substrates with pH levels ranging from 4 to 10.

The Iberian rock lizard, *Lacerta monticola cyrenni*, (the species selected as a model), is a medium-sized lizard inhabiting mountain areas of Central Spain which is susceptible to the affects of acid deposition. During the

early summer, females deposit 4 to 10 eggs in nests burrowed in the soil in rocky or grassy areas. It is a vulnerable species with a reduced and fragmented distribution and is susceptible to a number of environmental stresses (Pérez-Mellado, 1997; Barbadillo *et al.*, 1999).

### MATERIALS AND METHODS

#### EGG COLLECTION

We collected 10 gravid Iberian rock lizard females (*Lacerta monticola cyrenni*) from the Gredos Mountains (Avila, Spain), during the last week of June 2001. Females were collected in areas where they were abundant. They were housed individually in 30 L plastic containers in the laboratory at approximately 26 °C and were exposed to daylight, allowing some exposure to UV radiation. The bottom of the containers was filled with 8 cm of sand that was watered regularly. Dechlorinated tap water was always available. Females were fed with living *Tenebrio* larvae *ad libitum*, previously dusted with multivitamin powder. Egg laying took place after a maximum of 15 days in captivity. All females laid an average of 6.2 eggs per clutch. Immediately after egg-laying, the eggs were extracted from the sand, cleaned with a soft brush, marked individually by a number on the eggshell with a graphite pencil, weighed on a digital scale ( $\pm 0.01$  g) and measured with a caliper ( $\pm 0.1$  mm). Before the beginning of the experiment, the eggs were incubated at 26 °C in wet sand. After egg laying, females were released to the same places where they had been collected.

#### EXPERIMENTAL PROCEDURES

The effects of substrate pH on lizard egg incubation were tested by incubating single eggs in wet vermiculite

at the selected substrate pHs ranging from 4 to 10. We used vermiculite as substrate to reduce pH variation. One fertile egg from each clutch was randomly assigned to each of five pH treatments (4, 5, 7, 9 and 10) in a block design that controlled the effect of the intra-clutch variability. All eggs began experimental incubation on the same day. The experiment was conducted in July and August (2001) in an incubation chamber at 26 °C (Sanyo Incubator MIR 52). The selected values for temperature and water potential fall within the ranges of values measured for natural nests at the collection site. The experiment was conducted until the eggs hatched.

The fifty eggs were individually incubated inside plastic containers (110 ml) filled with 100 ml of sterile vermiculite. Selected substrate pHs were obtained and adjusted by adding concentrated solutions of HCl or NaOH to the vermiculite. These acid solutions were made by adding concentrated HCl or NaOH to 50 ml of distilled water. Then, the substrate of each container was watered with distilled water until a water potential of 150 KPa was reached (following the methodology proposed by Packard & Packard (1988)). Eggs were added to the containers after selected pH and water potential were obtained. The substrate covered the eggs completely. The containers were covered with lids to minimize evaporation. To control the substrate pH, we introduced into the incubator an additional container for each pH treatment with the wet vermiculite at the corresponding pH. The pH of the wet vermiculite before being placed in the experimental containers, and of the wet vermiculite in the enclosures without eggs, was measured periodically using a portable pH meter (Handylab 1 BNC, Schott-Geräte GmbH, Germany). In order to avoid substantial alterations of the selected pH values, substrates were replaced every 8 to 10 days with new substrates at the selected pH.

At the beginning of the exposure to the pH treatments and when the substrate was replaced, we recorded the survival rate and the external shape and aspect of eggs. We also weighed them on a portable digital scale to the nearest 0.01g. Immediately after hatching, we measured hatchling snout-vent length (SVL) and mass. We also checked for morphological or behavioural alterations. Hatchlings were housed in 30 L plastic containers in the laboratory at approximately 26 °C under natural light and were fed with crickets previously dusted with vitamin powder.

To determine whether substrate pH during incubation had an effect on hatchling locomotor abilities, we measured the running speed of each individual within the first 24 hr of hatching. All hatchlings had the tail intact. Hatchlings were forced to run a distance of 1m by chasing them by hand, following a simplified version of the methodology proposed by Huey *et al.* (1981). The track was constructed of cardboard and was 120 cm long with vertical walls 30 cm high which were positioned 20 cm apart; the floor was lined with filter paper. To calculate the running speed, we considered the time that hatchlings took to displace 100 cm, excluding the first and the last 10 cm of the track. Running times were re-

corded with a stopwatch to the nearest 0.1s. We tested each hatchling twice and considered the mean running speed for each individual. Between each trial, hatchlings were permitted to rest for 2 min. At the end of the experiment, hatchlings were released in the area where females had been collected.

#### ANALYSIS OF DATA

To determine whether substrate pH has an overall effect on egg incubation, we used analysis of covariance (ANCOVA), considering as dependent variables: final egg mass, time to hatching, hatchling mass, SVL, tail length and running speed. The covariate was the initial egg mass and the factor was the substrate pH. To determine the effect of substrate pH on embryo survival, we used the Chi-square test. We also used ANCOVA to determine the effect of pH on tail length and running speed considering hatchling SVL as covariate. We used the Tukey honest significant difference for post-hoc comparisons of means of ANCOVAs. All the assumptions of these analyses were previously verified.

#### RESULTS

ANCOVA indicated an effect of substrate pH on lizard egg incubation (Rao's  $R=5.261$ ;  $df=24,102$ ;  $P<0.001$ ). Nine eggs died during the experiment. Eight of them corresponded to two clutches. We did not find any external developmental abnormality on eggs or hatchlings. The tested levels of substrate pH had no effect on embryo survival ( $\chi^2=1.508$ ;  $df=4$ ;  $P=0.825$ ) and incubation duration (Table 1). On average, incubation lasted 35.6 days and hatching started after a mean time of exposure to pH treatments of 21 days. However, the substrate pH affected egg (Fig. 1) and hatchling mass (Fig. 2), tail length (Table 2) and running speed (Fig. 3). Post-hoc Tukey tests indicated that eggs incubated at a substrate pH of 4 and 10 absorbed less water and pre-hatching egg size and weight were lower than those at neutral pH (Table 1; Fig. 1). Post-hoc Tukey tests also indicated that hatchlings from eggs incubated at pH 4 were smaller (28%) than those incubated at pH 7 (Fig. 2). Similar results were obtained for SVL and tail length (Table 2). When we included hatchling SVL as a covariate in the ANCOVA analysis, the effect of

TABLE 1. Results of ANOVA that analyze the effect of substrate pH on initial egg mass and of ANCOVAs that analyze the effect of substrate pH on other parameters of Iberian rock lizard embryonic development using as the initial egg mass as a covariate.

Variable	df	F	P
Initial egg mass	4,41	0.02	0.999
Final egg mass	4,41	4.85	0.003
Incubation duration	4,36	1.27	0.266
Hatchling mass	4,36	17.34	<0.001
Hatchling SVL	4,36	2.32	0.075
Hatchling tail length	4,36	5.14	0.002
Running speed	4,36	2.96	0.032

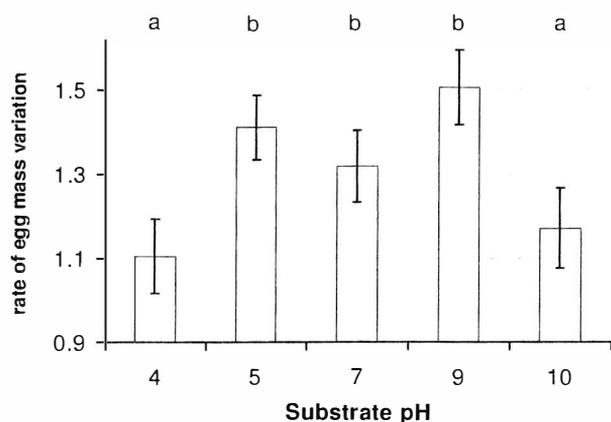


FIG. 1. Average rate of egg mass variation ( $\pm$  SE) of Iberian rock lizard eggs incubated at different substrate pHs. Letters over the bars indicate mean separation in post-hoc Tukey tests.

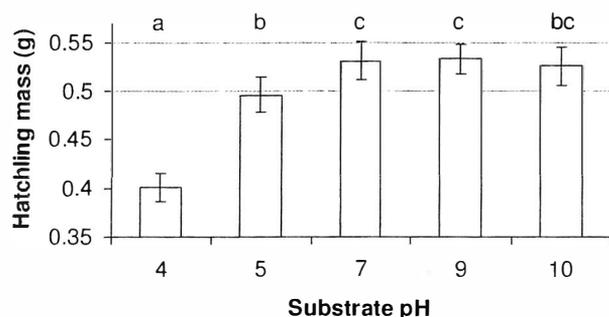


FIG. 2. Average mass ( $\pm$  SE) of Iberian rock lizard hatchlings from eggs incubated at different substrate pHs. Letters over the bars indicate mean separation in post-hoc Tukey tests.

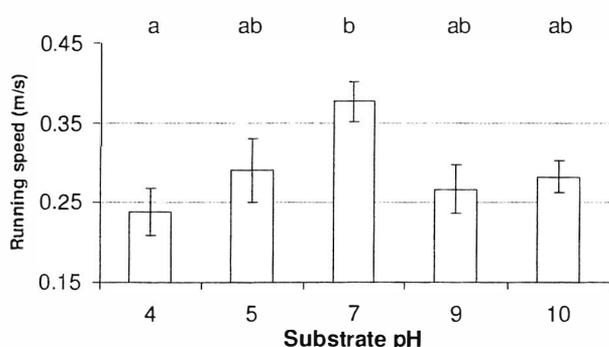


FIG. 3. Effect of substrate pH during flexible-shelled eggs' incubation on the running speed ( $\pm$  SE) of Iberian rock lizard hatchlings. Letters over the bars indicate mean separation in post-hoc Tukey tests.

TABLE 2. Mean ( $\pm$  SD) values of some characteristics of *Lacerta monticola* eggs and hatchlings exposed to different substrate pHs during incubation.

Substrate	4	5	7	9	10
Initial egg mass	0.435 (0.01)	0.435 (0.01)	0.43 (0.01)	0.432 (0.02)	0.432 (0.01)
Final egg mass	0.889 (0.04)	1.043 (0.03)	0.990 (0.03)	1.076 (0.04)	0.963 (0.04)
Incubation Duration	34.4 (0.54)	35.8 (0.57)	36.4 (0.57)	35.4 (0.65)	34.9 (0.60)
Dead eggs	1	3	1	2	2
Hatchling SVL	25.6 (0.41)	26.4 (0.43)	26.8 (0.43)	27.1 (0.49)	27.0 (0.46)
Hatchling tail length	39.9 (1.13)	42.8 (1.20)	46.1 (1.20)	45.6 (1.36)	43.6 (1.27)

substrate pH on hatchling mass was still significant ( $F_{4,36}=7.195, P<0.001$ ). Running speed varied significantly among treatments (Table 1) and was positively correlated with hatchling mass (Pearson correlation:  $r=0.467, F=19.92, P<0.001$ ). Embryos incubated at pH 4 and 5 generated hatchlings that ran significantly slower than controls (Fig. 3). The effect of soil pH on running speed was also significant when we included hatchling SVL as a covariate in the ANCOVA ( $F_{4,36}=3.181, P=0.025$ ).

## DISCUSSION

The results of this study demonstrate that flexible-shelled reptile eggs are sensitive to the pH of their nest environment. Low pH has a significant effect on egg water exchange, and also on hatchling size and running speed. These sublethal effects may influence the survival or success rates of juveniles. In all cases, pH had no effect on incubation duration and embryo survival. Basic pHs only slightly affected the egg mass variation during incubation. They had no effect on hatchling morphology. Eggs were exposed to the pH treatments during the last 60% of their embryonic development. Exposing eggs to extreme pHs during the full incubation period could increase the negative impacts on embryonic development.

Many factors affect the exchange of water between a reptilian egg and its environment (Packard & Packard, 1988; Ackerman, 1991). For example, the size of the egg, the proportion of the egg in contact with the substrate or the physical characteristics of the eggshell such as the porosity and rigidity can all determine how much water the egg can absorb or retain. Environmental factors such as the soil water potential or the temperature also influenced water exchange. We have found that the substrate pH may also affect the water absorption process that flexible-shelled reptile eggs experience during incubation. Acid and basic soil pHs alter the permeability of the eggshells or modify the availability of the soil water to the eggs in the nest environment.

Reptilian eggs and embryos are affected profoundly by the availability of water in their environment (Packard, 1991). Soil water potential in the nest may influence hatching success (Thompson, 1987; Packard, 1991). Moreover, embryos of flexible-shelled eggs incubated on wet substrates consume more oxygen, have a higher metabolic rate and grow more than those incubated in dry environments (reviewed in Packard, 1991).

Hydric conditions during incubation can also influence the hatchling locomotor performance of some reptiles (Miller *et al.*, 1987). In our experiment, we incubated all the eggs in wet substrates (-150 KPa) and enough water was available to guarantee optimal embryonic development. We demonstrate that substrate acidification causes similar effects on egg size, hatchling size and running speed as those of dry nest environments. Embryos in moist environments tend to remain in the egg longer before hatching than do embryos incubating in drier conditions (Packard, 1991). In moist environments, embryos grow for a longer period and hatch with larger sizes. However in our study, a decrease in the incubation period did not cause the observed effects related to substrate pH, because there were no differences in incubation duration among treatments.

Hatchlings from eggs incubated in acid substrates had lower body mass than controls and part of this difference was independent of hatchling SVL. One explanation for these results is the soil pH influence on the level of hydration of newly-hatched hatchlings. Other studies have shown that embryos incubated in dry substrates hatch less hydrated than those incubated on wet substrates (Packard, 1999). The level of hydration of hatchlings can explain the differences found on body mass and at the same time the absence of variability on body length among treatments. The state of desiccation has a strong influence on hatchling survival, especially in arid areas where hatchlings have difficulty accessing water sources. Large or well hydrated hatchlings usually survive better than smaller hatchlings during the neonatal period (Ferguson & Fox, 1984; Vleck, 1991). For example, large hatchlings may be better than smaller ones at emerging from dry nests, avoiding predators or capturing large prey (Packard, 1999).

Substrate pH had an effect on hatchling running speed. Hatchlings incubated at neutral pH were larger and ran faster than those incubated at acid pH and this difference was partially independent from hatchling SVL. The level of hydration may have an influence on locomotor performance. Alternatively, hatchlings incubated at acid pH may have developed less muscle mass. Lizards with a larger body size tend to have greater absolute stamina (Garland & Losos, 1994). Tail length could also influence hatchling locomotor performance. Short tails reduce running speed in some lizards (Ballinger *et al.*, 1979). Substrate acidification hampered hatchlings' capacity for running and hence reduced escape and foraging efficiency. Escape using fast movements is an important defensive behaviour for many lizard species and may be considered as an indicator of fitness (Bauwens & Thoen, 1981). Moreover, most reptiles have an intensive foraging strategy and they travel considerable distances, often at a very high running speed (Avery *et al.*, 1987). Decreased locomotor ability and increased energetic costs of locomotion due to egg incubation on acid substrates may influence the survival or success rates of juveniles.

Reptile species are declining on a global scale and environmental pollution has been suggested as one of the main causes (Gibbons *et al.*, 2000). Reptiles are exposed to numerous environmental contaminants and many studies document their bioaccumulation in different tissues and eggs (Sparling *et al.*, 2000). Most studies on reptile ecotoxicology have been conducted on turtles and crocodylians, but there are very few studies conducted on snakes or lizards. On the other hand, very few experiments document the effects and the dose-effect relationship of pollutants on reptiles (Kleinow *et al.*, 1999; Sparling *et al.*, 2000; Campbell & Campbell, 2002). Finally, it is assumed that the accumulation of toxicants or any other toxic effect on eggs originates from maternal transference during egg formation. There is very little evidence indicating that environmental pollution can have a direct impact on egg incubation. For example, oil applied to the external surface of bird eggshells produced high embryo mortality, but partial sealing of the shell and reduced gas exchange could be the cause of the death (Jessup & Leighton, 1996). Another study documents that organochlorine contaminants may pass through the eggshell of snake eggs incubated in contaminated nests (Cañas & Anderson, 2002). Our study documents the impact of soil pH on embryonic development of reptiles with flexible-shelled eggs. Acid rain, dry deposition or other sources of soil acidification could be contributing to the decline of reptiles with flexible-shelled eggs. Though there has been a significant reduction in anthropogenic acid deposition from the '60s, many aquatic and terrestrial ecosystems are still showing a very slow recovery or no recovery at all (Likens *et al.*, 1996; Alewell *et al.*, 2000).

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#### REFERENCES

- Ackerman, R. A. (1991). Physical factors affecting the water exchange of buried reptile eggs. In *Egg incubation: its effects on embryonic development in birds and reptiles*, 193-211. Deeming, D. E. and Ferguson, M. W. J. (Eds). Cambridge: Cambridge University Press.
- Alewell, C., Manderscheid, B., Meesenburg, H., Bittersohl, J. (2000). Is acidification still an ecological threat? *Nature* **407**, 855-857.
- Avery, R. A., Mueller, C. F., Smith, J. A. & Bond, D. J. (1987). The movement patterns of lacertid lizards: speed, gait and pauses in *Lacerta vivipara*. *Journal of Zoology (London)* **211**, 47-63.
- Ballinger, R. E., Nietfeldt, J. W. & Krupa, J. J. (1979). An experimental analysis of the role of the tail in attaining

- high running speed in *Cnemidophorus sexlineatus*. *Herpetologica* **35**, 114-116.
- Barbadillo, L. J., Lacomba, J. I., Pérez-Mellado, V., Sancho, V. & López-Jurado, L. F. (1999). *Anfibios y reptiles de la Península Ibérica, Baleares y Canarias*. Barcelona: Geoplaneta.
- Bauwens, D. & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology* **50**, 733-743.
- Campbell, K. R. & Campbell, T. S. (2002). A logical starting point for developing priorities for lizard and snake ecotoxicology: a review of available data. *Environmental Toxicology and Chemistry* **21**, 894-898.
- Cañas, J. E. & Anderson, T. A. (2002). Organochlorine contaminants in eggs: the influence of contaminated nest material. *Chemosphere* **47**, 585-589.
- Carcamo, H. A. & Parkinson, D. (2001). Localized acidification near sour gas processing plants: are forest floor macro-invertebrates affected? *Applied Soil Ecology* **17**, 199-213.
- Ferguson, G. W. & Fox, S. F. (1984). Annual variation of survival advantage of large juvenil side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**, 342-349.
- Gaerdenfors, U., Walden, H. W. & Waereborn, I. (1995). Effects of soil acidification on forest land snails. *Ecological Bulletin – Swedish National Science Research Council* **44**, 259.
- Garland, T. Jr. & Losos, J. B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In *Ecological morphology integrative organismal biology*, 240-302. Wainwright, P. C. and Reilly, S. M. (Eds). Chicago: University of Chicago Press.
- Gibbons, J. W., Scott, D. E., Ryan, T. J., Buhlmann, K. A., Tuberville, T. D., Metts, B. S., Greene, J. L., Mills, T., Leiden, Y., Poppy, S. & Winne, C. T. (2000). The Global decline of reptiles, déjà vu amphibians. *Bioscience* **50**, 653-666.
- Graveland, J. & Van Der Wal, R. (1996). Decline in snail abundance due to soil acidification causes eggshell defects in forest passerines. *Oecologia* **105**, 351-360.
- Huey, R. B., Schneider, W., Erie, G. L. & Stevenson, R. D. (1981). A field-portable racetrack and timer for measuring acceleration and speed of small cursorial animals. *Experientia* **37**, 1356-1357.
- Jessup, D. A. & Leighton, F. A. (1996). Oil pollution and petroleum toxicity to wildlife. In *Noninfectious diseases of wildlife*, 141-156. Fairbrother, A., Locke, L. N. and Hoff, G. L. (Eds). Ames: Iowa State University Press.
- Killham, K. (1994). *Soil ecology*. Cambridge: Cambridge University Press.
- Kleinow, K., Baker, J., Nichols, J., Gobas, F., Parkerton, T., Muir, D., Monteverdi, G. & Mastrodome, P. (1999). Exposure, uptake, and disposition of chemicals in reproductive and developmental stages of selected oviparous vertebrates. In *Reproductive and developmental effects of contaminants in oviparous vertebrates*, 9-112. Di Giulio, R. T. and Tillitt, T. (Eds). Pensacola: SETAC Press.
- Likens, G. E., Driscoll, C. T. & Buso, D. C. (1996). Long-term effects of acid rain: response and recovery of a forest ecosystem. *Science* **272**, 244-246.
- Miller, K., Packard, G. C. & Packard, M. J. (1987). Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *Journal of Experimental Biology* **127**, 401-412.
- Ormerod, S. J. & Rundle, S. D. (1998). Effects of experimental acidification and liming on terrestrial invertebrates: implications for calcium availability to vertebrates. *Environmental Pollution Serie B, Chemist and Physics* **103**, 183-192.
- Overall, K. L. (1994). Lizard egg environments. In *Lizard ecology: historical and experimental perspectives*, 51-72. Vitt, L. J. and Pianka, E. R. (Eds). New Jersey: Princeton University Press.
- Packard, G. C. (1991). Physiological and ecological importance of water to embryos of oviparous reptiles. In *Egg incubation: its effects on embryonic development in birds and reptiles*, 213-228. Deeming, D. E. and Ferguson, M. W. J. Cambridge: Cambridge University Press.
- Packard, G. C. (1999). Water relations of chelonian eggs and embryos: is wetter better? *American Zoologist* **39**, 289-303.
- Packard, G. C. & Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In *Biology of the Reptilia, Vol. 16-Ecology B, Defense and life history*, 523-606. Gans, C. and Huey, R. B. (Eds). New York: C. Alan R. Liss Inc.
- Pérez-Mellado, V. (1997). *Lacerta monticola* Boulenger, 1905. In *Fauna Ibérica*, vol. 10, 207-215. Salvador, A. (Ed). Madrid: CSIC.
- Reuss, J. O. & Johnson, D. W. (1986). *Acidic deposition and the acidification of soils and waters*. New York: Springer.
- Rubin, H., Narkis, N. & Carberry, J. (1998). *Soil and aquifer pollution*. Heidelberg: Springer-Verlag.
- Sparling, D. W., Linder, G. & Bishop, C. A. (2000). *Ecotoxicology of amphibians and reptiles*. Pensacola: SETAC Press.
- Thompson, M. B. (1987). Water exchange in reptilian eggs. *Physiological Zoology* **60**, 1-8.
- Vleck, D. (1991). Water economy and solute regulation of reptilian and avian embryos. In *Egg incubation: its effects on embryonic development in birds and reptiles*, 245-259. Deeming, D. E. and Ferguson, M. W. J. (Eds). Cambridge: Cambridge University Press.
- Yaron, B., Calvet, R. & Prost, R. (1996). *Soil Pollution Processes and Dynamics*. Heidelberg, Springer-Verlag.



## POST-METAMORPHIC GROWTH, SEXUAL MATURATION AND BODY SIZE DIMORPHISM IN THE SKIPPER FROG, *EUPHLYCTIS CYANOPHLYCTIS* (SCHNEIDER)

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Post-metamorphic growth to sexual maturity was studied in a tropical frog *Euphlyctis cyanophlyctis* that breeds all the year round, in large outdoor terraria. Sexual size dimorphism (SSD) was studied in both a natural population and those reared in outdoor terraria. The growth rate was high in the first 2-3 months in both sexes, but subsequently declined, reaching a plateau following sexual maturation. The growth rate was high during summer and low during winter, and its pattern paralleled the changes in the ambient temperature. The males matured between 3-6 months depending upon their growth rates; at this stage they developed vocal sacs and spermatozoa and engaged in calling. The females matured between 8-11 months of age and responded to injections of progesterone by producing mature eggs. Fecundity was positively correlated to snout-vent length (SVL)/body mass. The males and females attained maturity when they reached a critical minimum SVL of ~42 mm and ~55 mm respectively. Mean adult body size (SVL and body mass) of females collected from nature was  $67.0 \pm 0.85$  mm and  $32.8 \pm 1.56$  g, and that of males was  $48.0 \pm 0.37$  mm and  $10.1 \pm 0.31$  g. Male to female size ratio (F/M) was 1.4 indicating SSD and a larger female size. In laboratory-reared specimens also, SSD was obvious at sexual maturity. A larger body size in females is due to delayed sexual maturity relative to the males. These findings suggest that in *E. cyanophlyctis* SSD is manifested primarily due to differences in the age at sexual maturity rather than an inherent difference in the post-metamorphic growth rate between the sexes.

*Key words:* age at maturity, amphibia, Anura, reproduction, sex ratio

### INTRODUCTION

In amphibians, regulation of larval period, larval growth, size at metamorphosis, post-metamorphic growth and survival rates may affect the age, body size and timing of sexual maturation. Therefore, pattern of growth is a key aspect in the life history of amphibians. Though post-metamorphic growth and adult body size are under genetic control, several proximate factors such as food, predator pressure and geographic distribution (latitude and longitude) modulate the growth rates and size at maturity within and between species (Hemelaar, 1988; Jørgensen, 1992; Augert & Joly, 1993; Leclair & Laurin, 1996; Claude *et al.*, 1999). Sexual maturation is a key element in the life history of any organism, as the age and size at which it occurs may influence reproductive fitness (Stearns, 1992; Bernardo, 1993; Adolph & Porter, 1996). Several biotic and abiotic factors influence age and size at sexual maturity in several ways (Stearns & Koella, 1986). Also, individuals within a population may differ in their growth patterns/trajectories and mature at different ages.

Age at sexual maturity varies widely among amphibian species, some maturing as early as six months and others as late as 5-6 years (Bastein & Leclair, 1992; Cherry & Francillon, 1992; Jørgensen, 1992; Marquez *et al.*, 1997; Gramapurohit *et al.*, 2004a). Generally, species inhabiting the tropics are known to grow fast and

mature earlier than the temperate species since environmental factors do not vary drastically, and are therefore presumed to promote growth year round (Duellman & Trueb, 1986; Jørgensen, 1992). Nevertheless, growth rate and age at sexual maturity are species-specific and there exists a trade-off between growth and sexual maturation.

Studies that deal with post-metamorphic growth, age and size at maturity and sexual size dimorphism (SSD) are mostly on anurans that are seasonal breeders from temperate regions (Woolbright, 1983, 1989; Ryser, 1988; Cherry & Francillon, 1992; Tucker, 1995; Katsikaros & Shine, 1997; Platz *et al.*, 1997; Sagor *et al.*, 1998; Monnet & Cherry, 2002). To the best of our knowledge, there are no such studies on frogs that breed year round. The skipper frog, *Euphlyctis cyanophlyctis* is distributed throughout India. In southern India it breeds year round with peak breeding activity coinciding with the southwest monsoon. Many aspects of reproduction such as gonadal cycles, kinetics of germ cell production, sites of gonadal steroidogenesis are well known for this species (Saidapur, 1989). However, other aspects of the life history are not known. Therefore, it was of interest to study the growth rate, age at maturity and SSD in *E. cyanophlyctis* reared in outdoor enclosures.

### MATERIALS AND METHODS

Tadpoles of *E. cyanophlyctis* ( $n=100$ ) at stage 45 (Gosner, 1960) with rudimentary tails were collected during monsoon season between 1-5 September 2000

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from a pond on the Karnatak University Campus, Dharwad (15°17' N, 75°3' E) to study post-metamorphic growth and sexual maturity. They were maintained in a cement cistern (100 × 100 × 100 cm) until metamorphosis. The tail resorbed within three days of their collection. Individual frogs were marked at metamorphosis by toe clipping (avoiding clipping of the thumbs so as not to interfere with clasping ability during mating). The metamorphs were then reared in outdoor terraria (300 × 100 × 100 cm) each housing 20 individuals. Each terrarium was provided with a pool of water on one side and some refugia on the other. The frogs were given food *ad libitum* daily (small fishes, various insects and their larvae).

The snout-vent length (SVL) and body mass for individual frogs were recorded regularly at monthly intervals and each group of frogs was interchanged among terraria to avoid any positional effects. SVL was recorded to the nearest 1 mm using a ruler by gently pressing the frogs against a hard surface. Body mass was recorded on an electronic balance after emptying the bladder. The frogs were gently blotted before weighing. Sex of the frogs was identified in retrospect when they matured. The males have external vocal sacs that are easily seen. Attainment of sexual maturity was ascertained by the presence of sperm in the testes of males, and spawning in the case of females. The females were induced to spawn by injecting with progesterone (2 mg/ml saline/frog). Number of eggs produced by each female indicated fecundity. The air temperature was recorded daily at 1400 hrs during the study period.

One hundred newly metamorphosed frogs from the same pond were also toe-clipped and their monthly growth in nature was recorded for comparison with that of laboratory reared frogs. The pond dried after four months and hence no further recordings could be made. Monthly growth rate for each frog was calculated by  $\ln(SVL_t/SVL_0)$  where  $SVL_t$  is the length at the end of a month while  $SVL_0$  is length at the beginning and for body mass by  $\ln(W_t/W_0)$  where  $W_t$  is the mass at the end of month and  $W_0$  at the beginning.

The SVL and body mass were recorded for adult male and female *E. cyanophlyctis* (n=196) collected at random during the breeding season in July 2000 to determine SSD and male to female ratio (MFR) as per Lovich & Gibbons (1992). The MFR was calculated as mean adult size of the females / mean adult size of the males.

Monthly variation in growth rate of individual frogs was compared using multivariate analysis of variance (MANOVA) followed by a *posteriori* Bonferroni test. Monthly changes in SVL, body mass and the growth rates between sexes were compared using Mann Whitney *U*-tests. The relationship between SVL or body mass and fecundity was determined using linear regression. Growth rates of SVL and body mass of males and females in natural population were compared with that of the laboratory reared individuals using *t*-tests. All statistical tests were performed using SPSS software.

RESULTS

GROWTH PATTERN

Pattern of growth of females and males reared from metamorphosis to 1 yr old (September 2000 to September 2001) is shown in Figs. 1-2. In general, *E. cyanophlyctis* metamorphosed at 12.6% of adult body mass. At metamorphosis, mean SVL and body mass of males and females was comparable in laboratory and natural populations. During the first three months after metamorphosis, growth was high in males but declined later. The growth curve reached a plateau in males around the 3<sup>rd</sup> month. In females, the period of growth before sexual maturity was longer. They grew faster than their male counterparts from the 3<sup>rd</sup> month onwards although growth rate was not significantly different be-

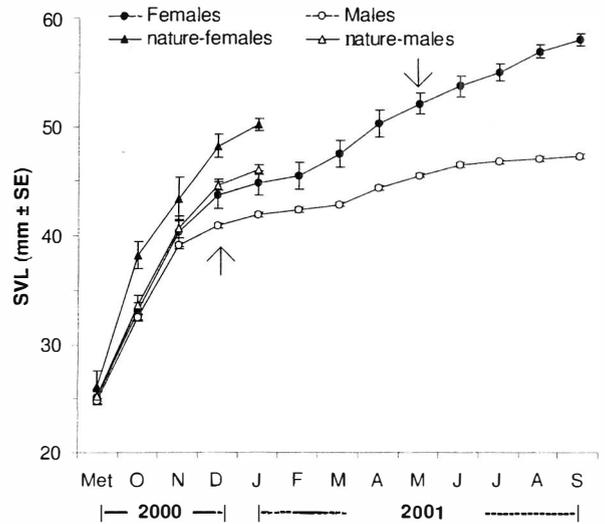


FIG. 1. Pattern of growth in SVL of *E. cyanophlyctis* reared in out-door terraria and from natural population (four months). Arrows indicate the onset of sexual maturity in laboratory-reared specimens. Met = metamorphosis

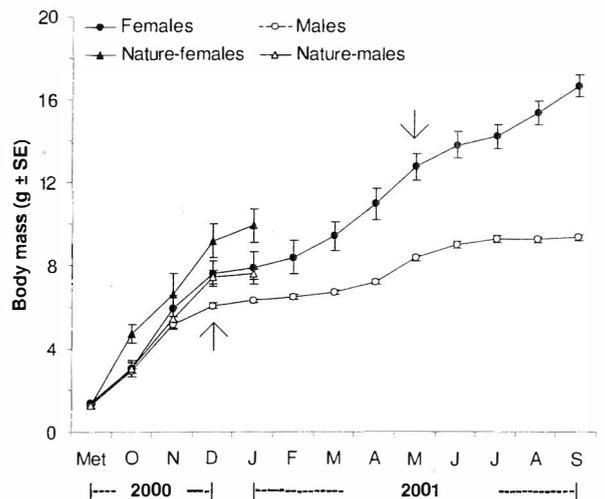


FIG. 2. Pattern of growth in body mass of *E. cyanophlyctis* reared in out-door terraria and from natural population (four months). Arrows indicate the onset of sexual maturity in laboratory-reared individuals. Met = metamorphosis

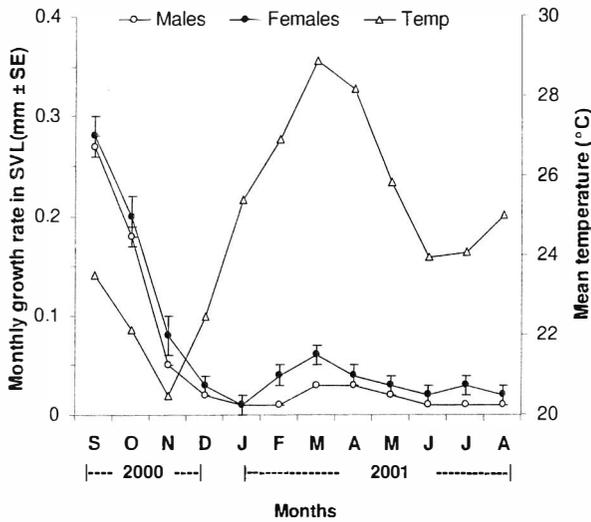


FIG. 3. Monthly variation in growth rate of SVL of *E. cyanophlyctis* in relation to ambient temperature from metamorphosis (September) to one year.

tween sexes during some months. Of the 100 frogs reared, 22 died due to unknown causes during the study period.

The growth pattern of frogs in a natural population (studied for four months) was similar to those reared in laboratory (Fig. 1-2). The recovery rate in the natural population became progressively low. At the end of the 4<sup>th</sup> month only 13 frogs could be recaptured of which eight were males with vocal sacs and five were females (without vocal sacs).

#### GROWTH RATE

Changes in the growth rate (SVL and body mass) in both sexes closely followed the changes in the ambient temperature (Figs. 3-4).

**Males.** The pattern of changes in growth rate of both SVL and body mass was similar (Figs. 3-4). Monthly growth rate of SVL ( $F_{11,804}=262.2$ ,  $P<0.0001$ ) and body mass ( $F_{11,804}=193.82$ ,  $P<0.0001$ ) varied significantly. It was highest in the 1<sup>st</sup> month of metamorphosis (September) but declined significantly in subsequent months up to December-January (Table 1). There was a significant rise in growth rate in March, which peaked in April-May (Table 1). Between June-August, rate of growth declined significantly.

**Females.** Monthly variation in the growth rates of SVL ( $F_{11,132}=47.67$ ,  $P<0.0001$ ) and body mass ( $F_{11,132}=31.43$ ,  $P<0.05$ ) was significant and followed a similar pattern (Figs. 3-4). Growth rates of SVL and body mass were highest in the first month of metamorphosis (Table 1) following which it declined gradually until December-January. From January-February onwards there was a significant rise in the growth rate until May followed by a steady decline until August.

Except for the first two months, the overall rate of growth of SVL ( $U=41266$ ,  $n_1=67$ ,  $n_2=11$ ,  $P<0.0001$ ) and body mass ( $U=42944$ ,  $n_1=67$ ,  $n_2=11$ ,  $P<0.0001$ ) of laboratory-reared frogs over a period of one year varied significantly between the sexes. The growth rate was

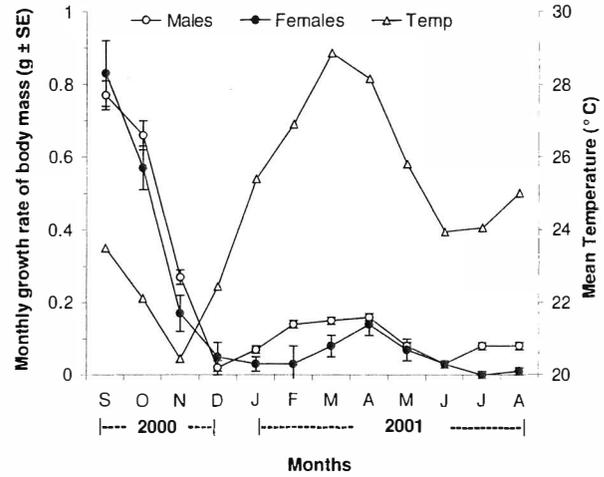


FIG. 4. Monthly variation in growth rate of body mass of *E. cyanophlyctis* in relation to ambient temperature from metamorphosis (September) to one year.

generally higher in females than in males from the 3<sup>rd</sup> month onwards though it was not statistically significant in some months.

Although growth rates were slightly higher in frogs in the natural population than in laboratory-reared frogs, these differences were not significant in either sex. (male SVL:  $t=0.80$ ,  $df=1$ ,  $P>0.05$ ; male body mass:  $t=0.57$ ,  $df=1$ ,  $P>0.05$ ; female SVL:  $t=0.49$ ,  $df=1$ ,  $P>0.05$ ; female body mass:  $t=0.62$ ,  $df=1$ ,  $P>0.05$ ).

**Age and size at sexual maturity.** In the laboratory, males attained sexual maturity between 3-6 months and females between 8-11 months after metamorphosis. Of these, 21 males matured three months after metamorphosis at a mean±SE SVL of  $42.6\pm0.25$  mm, and a mean mass of  $6.9\pm0.17$  g. Those that did not mature during the 3<sup>rd</sup> month ( $n=46$ ) were significantly smaller (SVL:  $40.7\pm0.32$  mm;  $t=4.81$ ,  $df=1$ ,  $P<0.001$ ; body mass:  $6.0\pm0.16$  g;  $t=3.97$ ,  $df=1$ ,  $P<0.001$ ) than those that matured. The females that matured at eight months, mean±SE SVL and mass at maturity were  $55.0\pm0.70$  mm and  $14.8\pm0.85$  g respectively ( $n=5$ ) while, those that did not ( $n=6$ ) were smaller in size (SVL:  $50.3\pm1.15$  mm; body mass:  $12.0\pm0.51$  g). All females matured by 11 months. In general, the fecundity (number of eggs) was positively correlated with both SVL and body mass. Irrespective of their age, the male and female frogs attained maturity when they reached SVL of ~42 mm and ~53 mm (±2mm) respectively (Fig. 5).

**Sexual size dimorphism.** The size frequency distribution of male and female *E. cyanophlyctis* collected from nature varied (Figs. 6-7). Mean±SE adult SVL and mass of females were  $67.2\pm0.85$  mm and  $32.8\pm1.56$  g respectively. Mean adult size (SVL and body mass) for males was  $48.0\pm0.37$  mm and  $10.1\pm0.31$  g. Male to female ratio (MFR: F/M) was 1.4. Among the laboratory reared frogs adult males were also always smaller than adult females of comparable age although they were of comparable size at metamorphosis (SVL:  $U=328$ ,  $n_1=67$ ,  $n_2=11$ ,  $P>0.05$ ; body mass:  $U=273.5$ ,  $n_1=67$ ,  $n_2=11$ ,

TABLE 1. The growth rate of SVL and body mass of *E. cyanophlyctis* reared in outdoor terraria along with MANOVA results.

Month	Sex	Growth rate (SVL)	MANOVA <i>t</i> -value	Growth rate (body mass)	MANOVA <i>t</i> -value
Sept	Male	0.27		0.83	
	Female	0.28		0.77	
Oct	Male	0.18	43.7, $P < 0.05$	0.57	37.3, $P < 0.05$
	Female	0.20	18.3, $P < 0.05$	0.66	13.3, $P < 0.05$
Nov	Male	0.05	26.1, $P < 0.05$	0.17	22.4, $P < 0.05$
	Female	0.08	11.1, $P < 0.05$	0.27	10.7, $P < 0.05$
Dec	Male	0.02	1.7, $P > 0.05$	0.05	0.2, $P > 0.05$
	Female	0.03	1.1, $P > 0.05$	0.02	1.5, $P > 0.05$
Jan	Male	0.01	5.9, $P < 0.05$	0.03	6.9, $P < 0.05$
	Female	0.01	3.9, $P < 0.05$	0.07	4.5, $P < 0.05$
Feb	Male	0.01	8.7, $P < 0.05$	0.03	7.7, $P < 0.05$
	Female	0.04	4.9, $P < 0.05$	0.14	4.5, $P < 0.05$
Mar	Male	0.03	8.4, $P < 0.05$	0.08	7.6, $P < 0.05$
	Female	0.06	2.3, $P < 0.05$	0.15	4.5, $P < 0.05$
Apr	Male	0.04	3.9, $P < 0.05$	0.14	4.9, $P < 0.05$
	Female	0.04	1.1, $P > 0.05$	0.16	4.5, $P < 0.05$
May	Male	0.03	5.5, $P < 0.05$	0.08	1.5, $P > 0.05$
	Female	0.03	2.9, $P < 0.05$	0.08	4.5, $P < 0.05$
Jun	Male	0.02	6.7, $P < 0.05$	0.07	5.6, $P < 0.05$
	Female	0.03	2.9, $P < 0.05$	0.03	4.5, $P < 0.05$
Jul	Male	0.01	9.5, $P < 0.05$	0.03	7.6, $P < 0.05$
	Female	0.03	4.1, $P < 0.05$	0.08	4.5, $P < 0.05$
Aug	Male	0.01	1.7, $P > 0.05$	0.01	9.4, $P < 0.05$
	Female	0.02	3.1, $P < 0.05$	0.08	4.5, $P < 0.05$

$P > 0.05$ ) and also during subsequent two months (Table 1). After attaining maturity in 3<sup>rd</sup> month, the growth rate of males declined. From then on they remained significantly smaller ( $U=15612$ ,  $n_1=67$ ,  $n_2=11$ ,  $P < 0.0001$ ) than females of comparable ages.

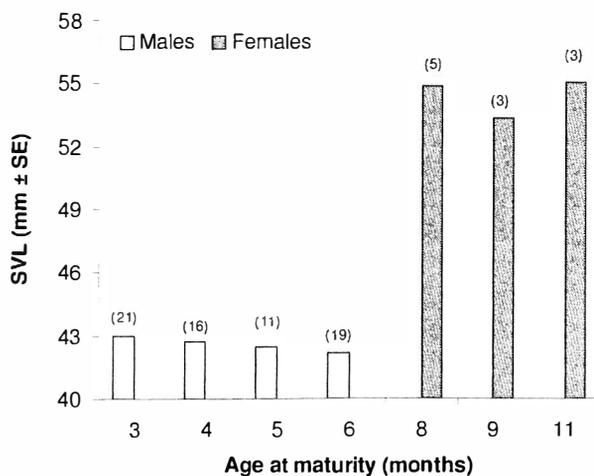


FIG. 5. The size and age at sexual maturity in *E. cyanophlyctis*. Note that both sexes matured when they reached a critical minimum size regardless of their age. (number in parentheses indicate the sample size).

## DISCUSSION

The size at metamorphosis is known to vary among different species of anurans. For instance, some bufonids metamorphose at a small size representing  $< 0.1\%$  of the adult body size while some ranids metamorphose at 20% of the adult body size (Werner, 1986). It is also

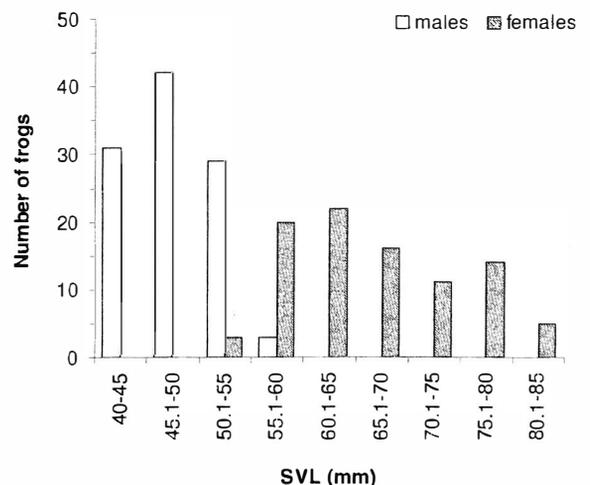


FIG. 6. The size (SVL) distribution of male and female *E. cyanophlyctis* in nature.

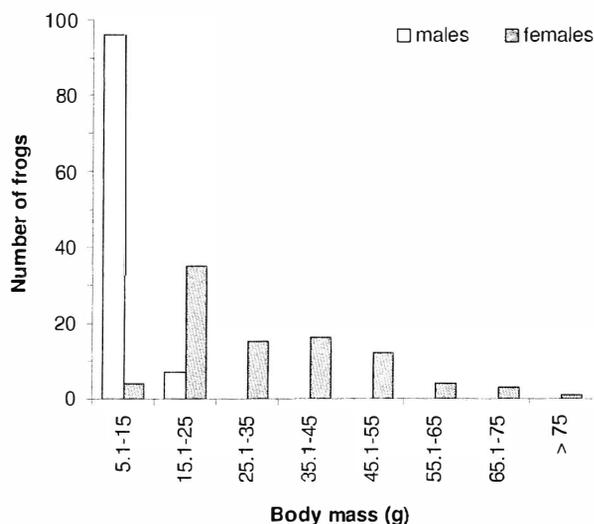


FIG.7. Distribution of body mass of male and female *E. cyanophlyctis* in nature.

well known that size at metamorphosis tends to be smaller in species breeding in temporary water bodies compared to those that breed in permanent water bodies (Werner, 1986). Therefore, in most aquatic species, size at metamorphosis tends to be larger than that of the terrestrial species (Werner, 1986). *Euphlyctis cyanophlyctis* is an aquatic species having smaller adult size and breeds in relatively permanent water bodies. *Hoplobatrachus tigerinus* a sympatric ranid is relatively terrestrial and has larger adult size and breeds in temporary ponds. Of these two *E. cyanophlyctis* metamorphosed at a larger size (12.6% of adult body mass) than *H. tigerinus* (0.4% of its mean adult mass; Gramapurohit *et al.*, 2004b). The present findings are in agreement with those reported for other temperate species and they corroborate the views of Werner (1986).

The fact that the growth rate of *E. cyanophlyctis* was similar in frogs reared in the laboratory and those from nature – at least during the first four months following metamorphosis – suggests near optimal conditions for growth in laboratory outdoor terraria. An initial period of rapid growth followed by a rapid decline as seen in *E. cyanophlyctis* is characteristic of post-metamorphic growth among most anurans (Jørgensen, 1992). In many temperate anurans, seasonal growth is influenced mainly by temperature (Jørgensen, 1992) with a few exceptions (Clarke, 1974). The fact that growth rates (SVL and body mass) in *E. cyanophlyctis* paralleled the ambient temperature (though winter is not severe in southern India) suggests that seasonal growth is not a characteristic feature of only temperate species and that it may be encountered in tropical species as well. Apparently, both internal and environmental factors appear to regulate seasonal growth (Jørgensen, 1992).

There is no general trend with regard to age at maturity between sexes in anurans. In some species both males and females attain sexual maturity at the same age (Francillon *et al.*, 1984; Acker *et al.*, 1986; Esteban, 1990) while in others males attain early maturity (Cherry &

Francillon, 1992; Marquez *et al.*, 1997). Interestingly, in *Pelobates cultripes* and *Rana perezi*, males are known to mature later than the females (Talavera, 1989; Docampo & Milagrosa, 1991). Unlike many temperate anurans, both sexes of *E. cyanophlyctis* attain sexual maturity in the first year itself and males attain sexual maturity much earlier than the females. In *Rana curtipes* (endemic to Western Ghats of southern India) males and females also attain sexual maturity in the first year, within six months of metamorphosis (Gramapurohit *et al.*, 2004a). To the best of our knowledge, the present study on *E. cyanophlyctis* is the second report documenting early attainment of sexual maturity following metamorphosis among anurans. In an earlier study, female *E. cyanophlyctis* was reported to attain maturity in the second year based on skeletochronological observations (Kulkarni & Pancharatna, 1996). Attainment of sexual maturity in the first year provides unequivocal proof regarding the age at which females attain sexual maturity in *E. cyanophlyctis*. It also suggests that conclusions based on mere skeletochronological observations may not be reliable. Further, in *E. cyanophlyctis* age at maturity is related to size rather than the age, as in the temperate-zone salamander *Triturus cristatus* (Sinsch *et al.*, 2003). On the other hand, in *H. tigerinus* sexual maturity was related to age (Gramapurohit *et al.*, 2004b) rather than size. Therefore, dependency on age and size for attaining sexual maturity appears to be species-specific and needs to be interpreted with due caution. An inherent variation in growth rate among individuals of male and female *E. cyanophlyctis* population is in agreement with the earlier reports on anurans (Halliday & Verrell, 1988). This is largely because of a variation in the inherent growth potential of different individuals to utilize resources before sexual maturity as suggested by Halliday & Verrell (1988). However, the present study reveals that attaining a critical SVL is crucial in attaining sexual maturity in male and female *E. cyanophlyctis*, a life history trait that might have been fixed in its phylogenetic history.

Most amphibians exhibit SSD with females being larger in about 90% of the species while, in the other 10%, males are either larger or no SSD exists (Shine, 1979, 1989, 1990). However, post-metamorphic growth rate and age at sexual maturity, the two important factors that affect or control SSD have received little attention (Arak, 1988; Monnet and Cherry, 2002). The present study on *E. cyanophlyctis* shows that SSD occurs due to differences in the post metamorphic growth period between the sexes before sexual maturity. In fact, male and female *E. cyanophlyctis* are of comparable size at metamorphosis but a trade-off between body growth and attainment of sexual maturity is dissociated between the sexes. The males attain maturity as early as three months and all individuals mature by six months depending upon their growth rates. On the other hand, female reproduction necessitates energy storage for production of yolk-laden eggs. Therefore, female *E. cyanophlyctis* grow for a longer period (8-11 months) to

attain a critical minimum size and then attain sexual maturity. Production of ovulatory sized eggs requires 3-4 months (Pancharatna & Saidapur, 1985). Thus, attainment of proper body size and delayed maturity help in regulating fecundity vis-à-vis fitness of female *E. cyanophlyctis*. Therefore, sexually mature females of *E. cyanophlyctis* are larger than mature males. Thus, in *E. cyanophlyctis*, differences in age at sexual maturity are chiefly responsible for SSD rather than differences in post-metamorphic growth rates *per se* between the sexes.

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#### REFERENCES

- Acker, P. M., Kruse, K. C. & Krehbiel, E. B. (1986). Aging *Bufo americanus* by skeletochronology. *Journal of Herpetology* **20**, 570-574.
- Adolph, S. C. & Porter, W. P. (1996). Growth, seasonality and lizard life histories: age and size at maturity. *Oikos* **77**, 267-278.
- Arak, A. (1988). Sexual dimorphism in body size: a model and a test. *Evolution* **42**, 820-825.
- Augert, D. & Joly, P. (1993). Plasticity of age at maturity between two neighbouring populations of the common frog (*Rana temporaria* L.). *Canadian Journal of Zoology* **71**, 26-33.
- Bastein, H. & Leclair, R. (1992). Ageing wood frogs (*Rana sylvatica*) by skeletochronology. *Journal of Herpetology* **26**, 222-225.
- Bernardo, J. (1993). Determinants of maturation in animals. *Trends in Ecology and Evolution* **8**, 166-173.
- Cherry, M. I. & Francillon, H. (1992). Body size, age and reproduction in the leopard toad, *Bufo pardalis*. *Journal of Zoology (London)* **228**, 41-50.
- Clarke, R. D. (1974). Postmetamorphic growth rates in a natural population of Fowler's toad, *Bufo woodhousei fowleri*. *Canadian Journal of Zoology* **52**, 1489-1498.
- Claude, M., Guyétant, R. & Elmberg, J. (1999). Variations in life history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology (London)* **249**, 61-73.
- Docampo, L. & Milagrosa V. M. (1991). Determinación de la edad en *Rana perezi* Seoane, 1885. Aplicación al análisis del crecimiento somático de poblaciones. Doñana, *Acta Vertebrata* **18**, 21-38.
- Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians*. New York: McGraw-Hill.
- Esteban, M. L. (1990). Evolución del género *Rana* en la península Ibérica: Estudio de la ariabilidad morfológica y genética del complejo *Rana temporaria* L. Thèse de doctorat, Université Madrid.
- Francillon, H., Barbault, R., Castanet, J. & Ricqlès, A. (1984). The biology of the desert toad *Bufo pentoni* age determination by the skeletochronological method population structure and dynamics. *Revue d' Ecologie la Terre et la Vie* **39**, 209-224.
- Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.
- Gramapurohit, N. P., Shanbhag, B. A. & Saidapur, S. K. (2004a). Post-metamorphic growth and sexual maturation in the bicoloured frog, *Rana curtipes* exhibiting delayed metamorphosis. *Amphibia-Reptilia* **25**, 445-451.
- Gramapurohit, N. P., Shanbhag, B. A. & Saidapur, S. K. (2004b). Growth, sexual maturation and body size dimorphism in the Indian bullfrog *Holobatrachus tigerinus* (Daud.). *Herpetologica* **60**, 414-419.
- Halliday, T. R. & Verrell, P. (1988). Body size and age in amphibians and reptiles. *Journal of Herpetology* **22**, 253-265.
- Hemelaar, A. (1988). Age, growth and other populations characteristics of *Bufo bufo* from different latitudes and altitudes. *Journal of Herpetology* **22**, 369-388.
- Jørgensen, C. B. 1992. Growth and reproduction In *Environmental physiology of the amphibians*, 439-466. Feder, M. E. and Burggren, W. W. (Eds). Chicago: University of Chicago Press.
- Katsikaros, K. & Shine, R. (1997). Sexual dimorphism in the tusked frog, *Adelotus brevis* (Anura: Myobatrachidae): the roles of natural and sexual selection. *Biological Journal of the Linnean Society* **60**, 39-51.
- Kulkarni, J. T. & Pancharatna, K. (1996). Age related changes in ovarian follicular kinetics in the Indian skipper frog, *Rana cyanophlyctis* (Schn.) *Journal of Bioscience* **21**, 699-710.
- Leclair, R. Jr., & Laurin, G. (1996). Growth and body size in populations of mink frogs, *Rana septentrionalis* from two latitudes. *Ecography* **19**, 296-304.
- Lovich, J. E. & Gibbons, J. W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth Development and Aging* **56**, 269-281.
- Marquez, R., Esteban, M. & Castanet, J. (1997). Sexual size dimorphism and age in the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Journal of Herpetology* **1**, 52-59.
- Monnet, M. J. & Cherry, M. I. (2002). Sexual size dimorphism in anurans. *Proceedings of Royal Society of London B* **269**, 2301-2307.
- Pancharatna, M & Saidapur, S. K. (1985). Ovarian cycle in the frog *Rana cyanophlyctis*: a quantitative study of follicular kinetics in relation to body mass, oviduct and fat body cycles. *Journal of Morphology* **186**, 135-147.
- Platz, J. E., Lathrop, A., Hofbauer, L. & Vradenburg, M. (1997). Age distribution and longevity in the Ramsey Canyon leopard frog, *Rana subaquavocalis*. *Journal of Herpetology* **31**, 552-557.
- Ryser, J. (1988). Determination of growth and maturation in the common frog, *Rana temporaria*, by skeletochronology. *Journal of Zoology (London)* **216**, 673-685.

- Sagor, E. S., Ouellet, M., Barten, E. & Green, D. M. (1998). Skeletochronology and geographic variation in age structure in the wood frog, *Rana sylvatica*. *Journal of Herpetology* **32**, 469–474.
- Saidapur, S. K. (1989). Reproductive cycles of amphibians. In *Reproductive cycles of Indian vertebrates*, 166–24. Saidapur, S. K. (Ed). New Delhi: Allied Press.
- Shine, R. (1979). Sexual selection and sexual size dimorphism in the amphibia. *Copeia* **1979**, 297–306.
- Shine, R. (1989). Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quarterly Review of Biology* **64**, 419–461.
- Shine, R. (1990). Proximate determinants of sexual differences in adult body size. *American Naturalist* **135**, 278–283.
- Sinsch, U., Lang, V & Wiemer, R. (2003). Dynamics of a crested newt metapopulation (*Triturus cristatus*) at a military training area (Schmittenhöhe, Koblenz): 3. Age structure. *Zeitschrift für Feldherpetologie* **10**, 229–244.
- Stearns, S. C. (1992). *The evolution of life histories*. Oxford, UK: Oxford University Press.
- Stearns, S. C. & Koella, J. C. (1986). The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for the age and size at maturity. *Evolution* **40**, 893–913.
- Talavera, R. (1989). Evolución de Pelobatidac y Pelodytidae (Amphibia: anura): morfología y desarrollo del sistema esquelético. Unpubl. Doctoral Thesis. Universidad Complutense. Madrid.
- Tucker, J. K. (1995). Early post-transformational growth in the Illinois chorus frog (*Pseudacris streckeri illinoensis*). *Journal of Herpetology* **29**, 314–316.
- Werner, E. E. (1986). Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *American Naturalist* **128**, 319–341.
- Woolbright, L. L. (1983). Sexual selection and size dimorphism in anuran amphibia. *American Naturalist* **121**, 110–119.
- Woolbright, L. L. (1989). Sexual dimorphism in *Eleutherodactylus coqui*: selection pressures and growth rates. *Herpetologica* **45**, 68–74.

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## BASKING COUNTS AS ABUNDANCE INDICES IN POND POPULATIONS OF *EMYS ORBICULARIS*

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The reliability of basking counts as indices of relative abundance for *Emys orbicularis* was tested in a pond system in central Italy. For different field conditions regression was carried out between basking indices and population size estimates obtained by capture and recapture methods. Regression was particularly significant in midday hours of sunny days from May to July, suggesting that in these conditions basking counts can be used as indices of population size.

*Key words:* Italy, terrapins, turtles, population estimates

### INTRODUCTION

The need for an improved methodological framework for population size estimates in freshwater turtles is increased by the threatened status of many species, calling for conservation measures based on good knowledge of population numbers and dynamics. This implies the development of standardized methods that must also maximize the ratio of results to effort, in terms of field work and statistical significance.

Population sizes of freshwater turtles are generally difficult to estimate in field studies. A first general problem, common to other vertebrate groups, is to identify the spatial limits of a population, in order to obtain values that refer to defined areas. Second, the natural complexity of the habitats preferred by many species – and their life-history traits – often make it difficult to observe or catch a large proportion of individuals.

Abundance estimates require long-term studies, taking into account different patterns of distribution in different habitats and possible metapopulation structures (see Burke *et al.*, 1995). Among the different approaches to the determination of population size, the most commonly used has been a sampling estimate based on CMR (capture-mark-recapture) methods (Graham, 1979). Nevertheless, individual marking has often been used to collect data on other topics (e.g. growth, movements), with abundance estimates as a secondary, unplanned result.

Relative abundance indices are simple tools for large-scale field sampling, although their use requires a preliminary calibration, i.e. knowledge of the relationships with true abundance estimates and significance levels of the models (Boulanger & Krebs, 1994; Stander, 1998).

In Italy the only native emydid is the European pond turtle *Emys orbicularis*, an endangered species included in national and regional Red Lists. Activity patterns of *Emys orbicularis* include large amounts of time spent basking in all seasons (Dal'Antonia *et al.*, 2001). The occurrence of this behaviour, together with a high de-

gree of site fidelity (Lebboroni & Chelazzi, 1991), provides the easiest conditions for detecting this species in the field.

The objectives of this work were to (1) test the reliability of basking counts as an abundance index, through a calibration with CMR estimates; and (2) define the seasonal and daily windows in which the index is best used, in order to obtain a method that could be used to survey a large number of ponds in a relatively short time.

### MATERIALS AND METHODS

Data were collected during long-term research on the ecology and the ethology of *Emys orbicularis* in central Italy. Field studies were carried out in the Natural Reserve Monte Rufeno, a hilly (300-600 m asl) area in Latium with a prevalence of woods dominated by turkey oak (*Quercus cerris*). Turtles live in small ponds (water surface 50-800 m<sup>2</sup>) scattered in the woods.

Turtles were captured by dip nets or by hand. After capture, turtles were individually marked with numbered tags to allow resighting from a distance. Inter-ponds movements were regularly performed in spring and summer by some individuals that used temporary pools near the permanent ones, where most turtles are found throughout the year. However, each turtle showed a high degree of fidelity to only one permanent pond in consecutive years (Lebboroni, 2000).

Detailed data on individual basking duration were collected for 20 adult and four subadult turtles in pond VH-1, with continuous observation from 08.00 hrs to 2000 hrs along three sunny days from May to July. Basking indices were obtained for six ponds by visual observation (using 10 × 40 binoculars) recorded from fixed points along pond banks. According to pond morphology and vegetation structure, for each pond one or two points were employed, in order to obtain a complete coverage of the whole surface. In the case of two observers, time concordance and detailed maps were used to avoid the possibility of double counting. Each session of observation (BC, basking count) lasted 20 mins, with the final variable BI, the basking index, scored as the total number of different turtles seen basking, regardless of the duration of the individual bout (a single event of

basking from emersion to immersion). In the analysis, data for sessions of 40 mins were also obtained, merging into two consecutive sessions. Only data on adult turtles were used, because of both low density of immatures in ponds and low detectability of these age classes.

A total of 120 hrs of observations were used for the analysis: each unit of 20 mins was allocated to one of the three corresponding periods of the day (0800-1200 hrs, 1200-1600 hrs, 1600-2000 hrs). In our study area this division was on average quite consistent with major changes in daily air temperature values. Observations relative to early spring (March-April), a period of low activity with some turtles still wintering, were tested separately from late spring/early summer (May to July), when these populations showed higher rates of activity (Lebboroni, 2000). For both seasons and for each daily period the BC was assigned to changeable or sunny weather according to conditions relative prevalence during the 20 mins: in completely overcast or rainy conditions no BC was performed. The interaction of temporal, seasonal, and meteorological factors gives a total of 24 possible categories (2 seasons  $\times$  2 weather  $\times$  3 daily periods  $\times$  2 count durations).

Population estimates for each pond (NP) from CMR analysis were obtained by Bailey's triple-catch method, considering the detection of individuals visually as capture (Caughley, 1980). The three sessions required by the method were carried out in early March (only marking), May (marking and recapturing) and late July (only recapturing). Analysis of the correlation of basking indices (BI) with population estimates (NP) was carried out using linear regression models (least squares fitting). All statistical analysis was carried out in S-Plus 4.5.

## RESULTS

A total of 84 adult turtles were marked in six ponds, with an overall estimate of 105 individuals: population sizes for single ponds ranged from 5 to 29, with narrow standard errors (Table 1). No significant relationship was found between turtle numbers and pond surface ( $r=0.6$ ,  $P>0.1$ , Spearman rank test).

The total daily time spent in basking was divided between several bouts, with turtles changing sites during

TABLE 1. Population estimates ( $\pm$ SE) of adult *Emys orbicularis* for six ponds. Number of marked turtles is the total for March and May sessions (see text). Total number of adult turtles observed is the total of marked and maximum number of unmarked individuals observed.

Pond code	No. of marked adult turtles	Total no. of adult turtles observed	Total estimate $\pm$ SE
DR-1	24	27	29 $\pm$ 3.6
VH-1	25	26	27 $\pm$ 2.2
DR-2	18	21	22 $\pm$ 1.7
VH-2	8	11	11 $\pm$ 3.1
PO-1	7	10	11 $\pm$ 2.8
DR-3	2	5	5 $\pm$ 1.8

the day. Average bout duration was not significantly different among males, females and subadults (65, 67 and 71 mins, respectively; Wilcoxon test,  $P>0.1$ ; Fig. 1).

Because of unpredictable weather, logistic conditions in the field, and avoiding use of small sample size ( $n<5$ ), linear models relating BI to NP were obtained for 14 out of the 24 possible categories. A significant regression (at least  $P<0.01$ ) was obtained for three conditions: (1) BC of 20 mins in sunny midday in May-July; (2) BC of 20 mins in sunny mornings in May-July; (3) BC of 40 mins in mornings with changeable weather in March-April (Fig. 2). The best fitting equation was obtained for the first linear model, with  $NP = 2.84 BI + 1.22$  ( $n=6$ ,  $r^2=0.89$ ,  $F=123$ ,  $P<0.0001$ ).

The lines for which the regression was significant show graphically the different relevance of the slope in the different periods. Computing NP from BI, a lower value of correction is required for midday hours during sunny days in late spring and early summer, meaning that under these conditions basking behaviour is performed by a larger fraction of the population.

## DISCUSSION

Few studies have specifically investigated the technical and statistical issues involved in field estimates of freshwater turtles (Ream & Ream, 1966; Bider & Hoeck, 1971; Frazer, 1990; Lindeman, 1990). Discussion has often involved the reliability of maintaining assumptions of these methods: particularly, the problem of unequal catchability seems difficult to avoid even after the application of different techniques for correcting bias (Koper & Brooks, 1998).

Population estimates by CMR in our study have proved reliable thanks to the intensive sampling effort. The high numbers of marked *Emys orbicularis* (75% of estimated adults) coupled with a good knowledge of the ponds allowed counts of turtles that were well correlated with true adult population sizes.

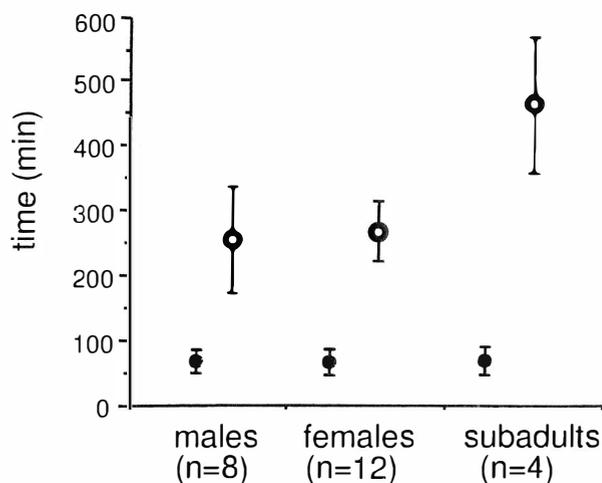


FIG. 1. Mean values ( $\pm$  SE) of basking bout duration (black circles) and total daily basking time (open circles) for adults and subadults in pond VH-1 for May to July. Sample sizes refer to the number of different turtles.

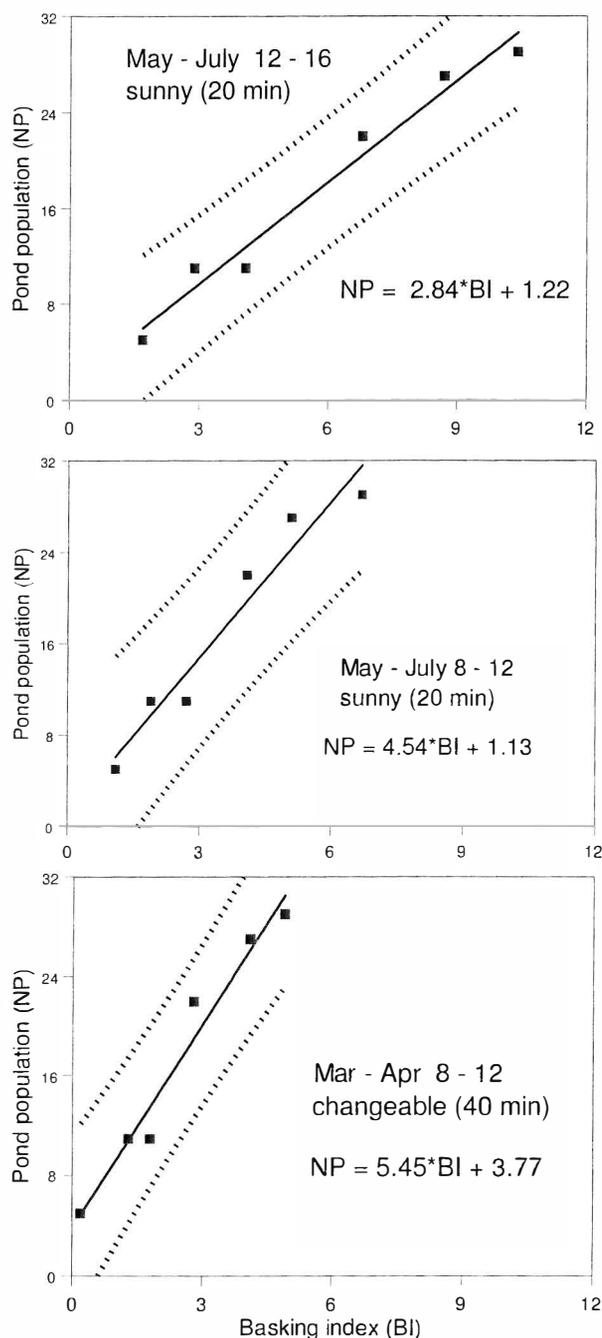


FIG. 2. A comparison of the significant regression lines (with confidence intervals at 95%) in different seasons, hours and weather conditions for all ponds ( $n=6$ ; points: average values of BI).

Concerning the values of indices, the similarity of basking patterns in males and females avoided bias due to intersexual differences. No significant differences were found between the sexes in total basking time, and similar values of average bout duration have been also reported for male and female *Chrysemys picta* (Lefevre & Brooks, 1995).

Environmental conditions in which a significant correlation of BI with NP was found were consistent with basking pattern reported for this species, in terms of seasonal and daily frequency (Rovero *et al.*, 2000).

For March-April, correlation was significant only for long sessions of observation: water temperature in early

spring may not be so uniform in all ponds as from May onwards, affecting basking behaviour.

Concerning the time spent making observations, no significant relationships were obtained for sessions lasting 40 mins in May-July. This could be explained by considering the average bout time of adults, because in a longer session the probability of including emersion and/or immersion is higher. Moreover, the effect of disturbance (by both unpredictable events and movements of the observer) could be more frequent, causing the immersion of turtles.

Midday hours with sunny weather from May to July showed the best conditions for assessment of population sizes in ponds. The value of the intercept of the regression line (1.22) seems ecologically meaningful, considering that single sessions of BC can overlook ponds with only 1-2 specimens.

The application of basking indices is limited to adult turtles not only for their higher detectability, but also because subadults are scarce in each pond, while turtles in age classes up to 3 yrs are spatially separated, living in swamps nearest to the nesting areas (Lebboroni & Chelazzi, 1999).

We are aware that the index we suggest has been tested on only a few ponds, with adult turtles ranging from 5 to 30, a small number when compared with larger populations living in canals and marshes of coastal central Italy (Zuffi *et al.*, 1999). Nevertheless, *Emys orbicularis* is widespread in habitats such as ponds in many areas of its European range (cfr. Servan, 1998). In peninsular Italy, because of pond location, difficult access and the general lack of ecological data for these areas, it is possible that ponds – especially those in hilly areas – can support a consistent number of small population of this species.

As with all other relative indices of abundance, the use of basking counts in ponds can be recommended as a monitoring scheme in two main situations: (1) at large spatial scales in similar habitats for a comparison of abundances; and (2) at small spatial scales for a detection of population trends in consecutive years. For both aspects there is an increasing need for a standard field methodology to apply at a national level, in order to carry out identification of endangered habitats and/or species suggested by European directives (i.e. 'Habitat' 92/43/CEE, including *Emys orbicularis*).

Further tests on the reliability of basking counts will assess the possibility of applying the method on a larger scale for this species.

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#### REFERENCES

- Bider, J. R. & Hoek, W. (1971). An efficient and apparently unbiased sampling technique for population studies of painted turtles. *Herpetologica* **27**, 481-484.

- Boulanger, J. & Krebs, C. J. (1994). Comparison of capture-recapture estimators of snowshoe hare populations. *Canadian Journal of Zoology* **72**, 1800-1807.
- Burke, V. J., Greene, J. L. & Gibbons, J. W. (1995). The effect of sample size and study duration on metapopulation estimates for slider turtles (*Trachemys scripta*). *Herpetologica* **51**, 451-456.
- Caughley, G. (1980). *Analysis of vertebrate populations*. Chichester: John Wiley & Sons.
- Dall'Antonia, L., Lebboroni, M., Benvenuti, S. & Chelazzi, G. (2001). Data loggers to monitor activity in wild freshwater turtles. *Ethology Ecology & Evolution* **13**, 81-88.
- Frazer, N. B., Gibbons, J. W. & Owens, T. J. (1990). Turtle trapping: preliminary tests of conventional wisdom. *Copeia* **1990**, 1150-1152.
- Graham, T. E. (1979). Life history techniques. In *Turtles: perspectives and research*, 73-96. New York: John Wiley & Sons.
- Koper, N. & Brooks, R. J. (1998). Population-size estimators and unequal catchability in painted turtles. *Canadian Journal of Zoology* **76**, 458-465.
- Lebboroni, M. (2000). Eco-etologia e demografia della tartaruga palustre europea *Emys orbicularis* (Linnaeus 1758) in un'area dell'Italia centrale. Tesi di Dottorato, Università di Firenze.
- Lebboroni, M. & Chelazzi, G. (1991). Activity patterns of *Emys orbicularis* L. (Chelonia Emydidae) in central Italy. *Ethology Ecology & Evolution* **3**, 257-268.
- Lebboroni, M. & Chelazzi G. (1999). Habitat use, reproduction and conservation of *Emys orbicularis* in a pond system in Central Italy. *Proceedings of the International Conference of the Pond Life Project*, 169-173. Maastricht, September 1998.
- Lefevre, K. & Brooks, R. J. (1995). Effects of sex and body size on basking behavior in a northern population of the painted turtle, *Chrysemys picta*. *Herpetologica* **51**, 217-224.
- Lindeman, P. V. (1990). Closed and open model estimates of abundance and tests of model assumption for two populations of the turtle, *Chrysemys picta*. *Journal of Herpetology* **24**, 78-81.
- Ream, C. & Ream, R. (1966). The influence of sampling methods on the estimation of population structure in painted turtles. *American Midland Naturalist* **75**, 325-338.
- Rovero, F., Lebboroni, M. & Chelazzi, G. (2000). Organizzazione spaziale e temporale dell'attività in una popolazione di tartaruga palustre europea *Emys orbicularis* (L.). *Atti del I Congresso Nazionale della Societas Herpetologica Italica* (Torino, 1996). Museo regionale Scienze naturali Torino 2000, 159-166.
- Servan, J. (1998). Ecological study of *Emys orbicularis* in Brenne (Central France). *Mertensiella* **10**, 245-252.
- Stander, P. E. (1998). Spoor counts as indices of large carnivore populations: the relationship between spoor frequency, sampling effort and true density. *Journal of Applied Ecology* **35**, 378-385.
- Zuffi, M. A. L., Odetti, F. & Meozzi, P. (1999). Body-size and clutch-size in the European pond turtle, *Emys orbicularis*, from central Italy. *Journal of Zoology, London* **247**, 139-143.

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## AMPHIBIAN AND REPTILE CONSUMPTION BY OTTERS (*LUTRA LUTRA*) IN A COASTAL AREA IN SOUTHERN IBERIAN PENINSULA

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Through the analysis of 1518 otter spraints we determined the importance of amphibians and reptiles in otter diet and the seasonal patterns of consumption of the different species in a heterogeneous coastal environment in southern Spain. Otters fed on a minimum of six amphibian and three reptile species. Amphibians were present in 13.2% and reptiles in 3.9% of the spraints analysed. Remains of amphibians and reptiles were significantly associated in otter spraints, but negatively associated with crayfish occurrences. Maximum reptile consumption occurred during the summer. Though terrapins have been rarely cited as otter prey, they were consumed more than twice as frequently as water snakes in the study area. The highest frequency of amphibians in the otter diet was recorded in late winter-spring surveys, coinciding with the spawning periods of most species. However, the frequency of amphibians remained high during summer months due to predation on the Iberian green frog (*Rana perezi*), a very aquatic frog that was almost the only amphibian species consumed in this season.

*Key words:* *Bufo bufo*, coastal otters, predation, Mediterranean streams, *Mauremys leprosa*, *Rana perezi*

### INTRODUCTION

The otter (*Lutra lutra*, Carnivora, Mustelidae) is a top predator in freshwater ecosystems. Featuring both anatomical and physiological adaptations for semi-aquatic living, the otter is specialized in obtaining virtually all its food in the water (Carss, 1995). Fish are the main prey of otters, and are preferentially consumed whenever readily available (Erlinge, 1968; Mason & Macdonald, 1986). However, otter diet is very variable (both temporally and geographically) and may incorporate most kinds of aquatic animals, including amphibians and reptiles, as well as crayfish and other crustaceans, aquatic insects, birds and mammals (Jędrzejewska *et al.*, 2001; Clavero *et al.*, 2003).

Amphibians are predated by the otter throughout the species' European range (Clavero *et al.*, 2003), constituting an important part of otter diet in many locations (Erlinge, 1972; Adrián & Delibes, 1987; Brzeziński *et al.*, 1993; Beja, 1996a; Sulkava, 1996). Reptiles are rarer in the otter's diet, since their consumption is usually restricted to lower latitudes (Ruiz-Olmo, 1995). However reptiles, specially water snakes (*Natrix* spp.), have been shown to be frequent otter prey in some Italian (Arcá & Prigioni, 1987) and Iberian studies (Simões-Graça & Ferrand de Almeida, 1983; López-Nieves & Hernando, 1984).

In this work we analyse the consumption of amphibians and reptiles by otters in the species' southernmost European location. Here, the diet of the otter has been described previously by Clavero *et al.* (2004). In particular we will try to answer the following questions: (1)

what species of amphibians and reptiles are consumed and in what proportions? (2) Are there seasonal patterns in the consumption of the different species?

### STUDY AREA

The study was conducted in the surroundings of Tarifa (Cádiz, Spain), the southernmost European town. The area occupied by otters is very heterogeneous, including sandy and rocky coastal stretches, estuarine areas and four main streams (Fig. 1). Mean annual precipitation ranges from 1300 to 620 mm, while mean annual temperature is around 17.5°C (Ibarra, 1993). Due to the oceanic influence, it is extremely rare that temperature falls below 0°C, and the mean minimum temperature in January is 11°C. More information on the study area's characteristics can be found in Clavero *et al.* (2004).

The area holds a very rich herpetofauna, with 20 reptile species – including four with semi-aquatic habits – and 10 amphibian species (Pleguezuelos *et al.*, 2002).

### METHODS

Otter diet was studied through the analysis of faeces (spraints). Otter spraints were collected in nine 600 m transects placed in lower and upper stretches of the four streams included in the study area and in the common estuary of two of them (Fig. 1). The different transects were classified as *lower* (coastal, numbers 2, 4, 5, 6 and 9 in Fig. 1) or *upper* (inland, numbers 1, 3, 7 and 8). Spraint collection was performed bimonthly from December 1999 to December 2001, with the exception of October 2001, due to heavy rains.

Spraint analysis followed standard procedures (Beja, 1997). Amphibian species were identified using the key for ilia bones by Felix & Montori (1986) and drawings of other bone structures provided by Boulenger (1897).

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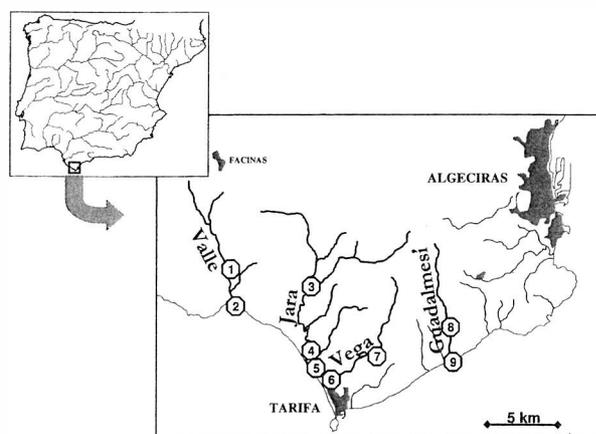


FIG 1. Map of the study area showing the nine transects in which otter spraints were collected.

Each prey type identified in a spraint was considered an occurrence, diet composition was then expressed either as Frequency of Occurrence (FO; number of occurrences of a certain prey type/number of spraints analysed) (Mason & Macdonald, 1986). Before being subject to statistical analysis, frequency data were arcsine transformed (Zar, 1984). The minimum number of individuals of a certain prey type present in a spraint was estimated from the number and position of ilia, tibia-fibulas and parasphenoid bones. Whenever these key structures did not appear in spraints, remains of a certain prey type were considered to belong to a single individual.

The association between amphibians and reptiles and other prey types was analysed using the  $\chi^2$  test. The prey types analysed were fish, crayfish (*Procambarus clarkii*) and insects. Eels (*Anguilla anguilla*) and freshwater fish (the pool of chub, *Squalius pyrenaicus*, and loach, *Cobitis paludica*) were also analysed separately, since these are the only three fish species which can be found far from the coast in the area (Clavero *et al.*, 2002). In coastal stretches other fishes, such as grey mullet (Mugilidae), flatfish (Soleidae) or wrasses (Labridae), are present and are important components of otter diet (Clavero *et al.*, 2004). Since coastal otters often feed in estuaries or in the open sea, where they are not supposed to find amphibians or reptiles, two different analyses of the association of prey types in spraints were performed. One was done considering all the spraints analysed, while in the second one we only considered spraints collected in upper stretches. The few samples that contained two or more pooled spraints were excluded from the association analyses.

## RESULTS

### AMPHIBIAN AND REPTILE OCCURRENCE IN OTTER DIET

A total of 1518 otter spraints were analysed (Table 1). Amphibian remains were identified in 13.2% of the spraints while reptiles were present in 3.9%. The FOs of amphibians in the different transects ranged from 4.8% to 54.3% and that of reptiles from 0.5% to 30.0%. The

FO of amphibian was significantly higher in upper stretches than in lower ones ( $t=2.9$ ,  $df=7$ ,  $P<0.05$ ). Though reptiles were also more frequently found in otter spraints in upper stretches, differences were not significant.

Overall, a minimum of 319 amphibian and 60 reptile individuals were identified (Fig. 2). Over 95% of the identified amphibians were anurans, while the urodeles were poorly represented in otter diet. More than a third of the amphibians predated by the otter were Iberian green frogs (*Rana perezi*). Iberian parsley frogs (*Pelodytes ibericus*), common toads (*Bufo bufo*) and stripeless tree frogs (*Hyla meridionalis*) constituted between 10% and 15% of the consumed individuals. Around 20% of the anuran individuals remained unidentified, as did the few urodeles that were found in spraints. Among reptiles, leprous terrapins (*Mauremys leprosa*) were the dominant prey. Around one third of the consumed reptiles were viperine snakes (*Natrix maura*), while only one lacertid – which remained unidentified – was found.

When the total number of spraints analysed was considered, amphibians and reptiles were shown to occur together in spraints more often than would be expected by chance (Table 2). Both amphibians and reptiles followed a similar pattern of association in spraints with another prey types, being positively associated with freshwater fish and insects and negatively associated with all fish included in otter diet. The occurrence of eels and crayfish in spraints was found to be independent of amphibians and reptiles. However, when only samples from upper stretches were considered, the patterns of association of the different prey types in spraints displayed clear changes. In freshwater habitats crayfish was the only prey type negatively associated both with amphibians and reptiles, while fish became independent of them. The positive association in spraints among amphibians, reptiles and insects remained significant in upper stretches.

### SEASONAL VARIATION IN AMPHIBIAN AND REPTILE CONSUMPTION

The overall analysis of the occurrence of amphibians and reptiles in the otter diet must be treated carefully, since there were clear seasonal variations in their consumption by otters (Fig. 3).

An increase in reptile consumption was observed from winter to summer reaching its peak in June in upper transects, and in August in lower ones. However, predation upon the two reptiles consumed by the otter in the area followed different seasonal patterns (Fig. 4).

TABLE 1. Yearly variation of the number of otter spraints analysed in lower and upper transects of the study area.

	Dec.	Feb.	Apr.	Jun.	Aug.	Oct.
Upper transects	132	53	116	109	78	18
Lower transects	344	165	164	144	84	123

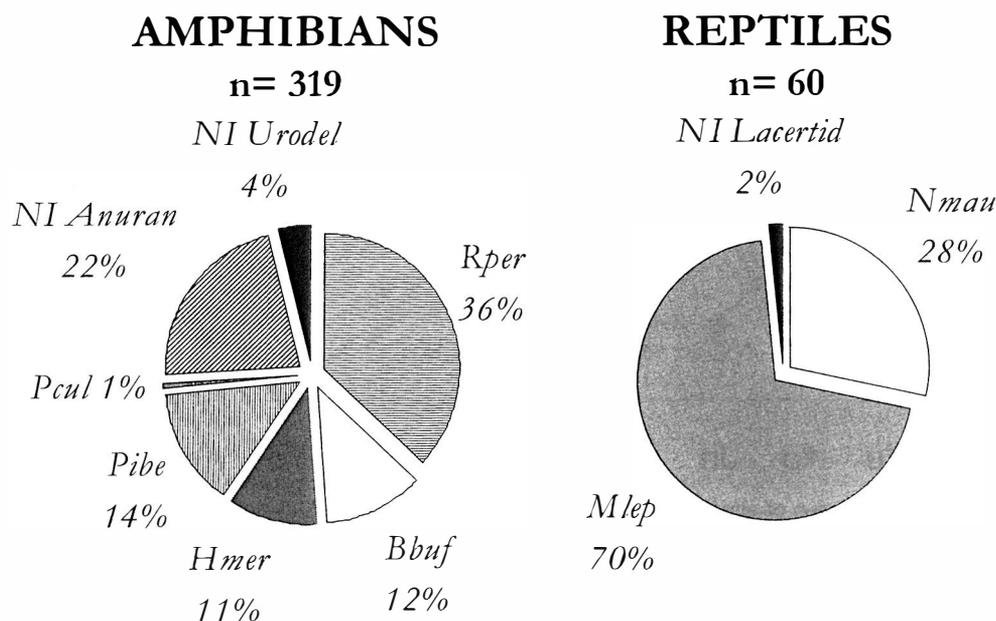


FIG. 2. Proportion of individuals of the different amphibian and reptile species identified. Amphibians: *Rper*, *Rana perezi*; *Bbuf*, *Bufo bufo*; *Hmer*, *Hyla meridionalis*; *Pibe*, *Pelodytes ibericus*; *Pcul*, *Pelobates cultripes*. Reptiles: *Nmau*, *Natrix maura*; *Mlep*, *Mauremys leprosa*.

Viperine snakes were consumed exclusively in spring-summer months with a marked peak in June. Consumption of terrapins showed a summer peak both in upper and lower transects, though we found their remains in otter spraints also during winter and spring. However there was a sharp decrease in terrapin occurrence in October.

Predation on amphibians reached its maximum between February and April, but was maintained at relatively high levels during the summer. This pattern was the result of the seasonal variation in consumption of the different anuran species (Fig. 5). Toads, stripeless tree frogs and Iberian parsley frogs were consumed almost exclusively during the breeding periods in late winter and the beginning of the spring. However, predation upon the Iberian green frog – the most frequent amphibian in the otter diet – reached its maximum in August, when it was almost the only amphibian consumed.

DISCUSSION

In the study area otters fed on a minimum of nine different amphibian and semi-aquatic reptile species. Though higher frequencies in otter diet have been previously reported for both amphibians and reptiles (i.e. Arcá & Prigioni, 1987; Brzeziński *et al.*, 1993), no other otter diet studies have yet recorded such diverse predation on these groups. The overall secondary role of amphibians and reptiles is undoubtedly influenced by the high proportion of marine and estuarine prey in otter diet in the area (Clavero *et al.*, 2004), since coastal otters rarely feed on non-fish prey (Jędrzejewska *et al.*, 2001).

The positive association of amphibians and reptiles in otter spraints suggests that they are frequently captured during the same foraging bouts. On the other hand, when only upper stretches were considered crayfish were negatively associated both with amphibians and reptiles. Crayfish are by far the most important freshwa-

TABLE 2. Association among different prey types in otter spraints assessed by  $\chi^2$  test (Pos- positively associated; Neg- negatively associated; Ind- independent occurrence in spraints). Results are shown separately for all the spraints analysed and for spraints collected in upper stretches only. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

ALL TRANSECTS (n=1470)						
	Fish (total)	Eels	Freshw. fish	Crayfish	Insects	Reptiles
Amphibians	Neg***	Ind	Pos***	Ind	Pos***	Pos***
Reptiles	Neg*	Ind	Pos**	Ind	Pos***	-
UPPER TRANSECTS ONLY (n=464)						
Amphibians	Ind	Ind	Pos**	Neg***	Pos***	Pos**
Reptiles	Ind	Ind	Ind	Neg***	Pos***	-

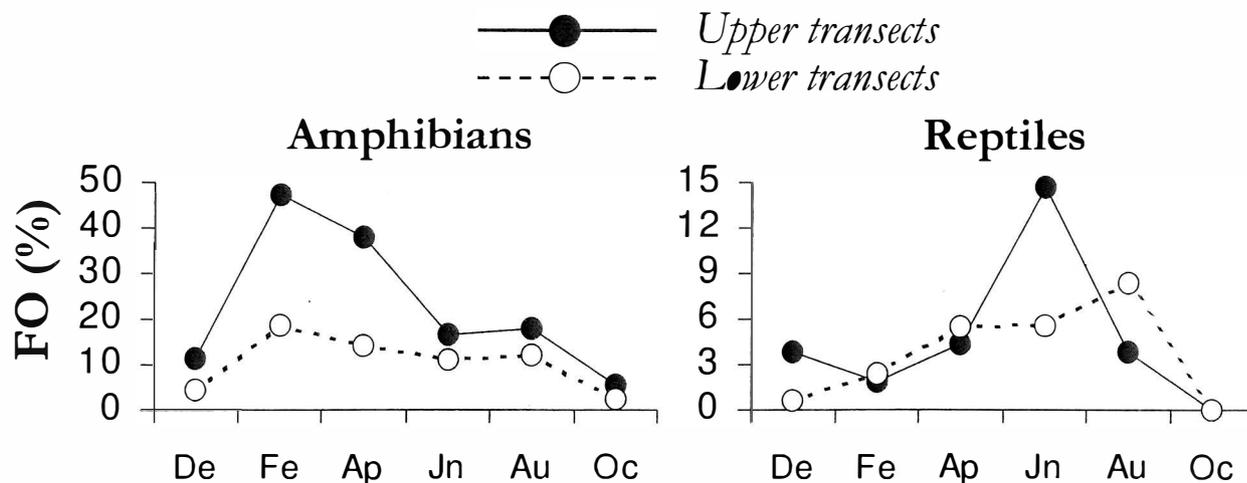


FIG 3. Yearly consumption of amphibian and reptiles by otters in upper and lower transects of the study area.

ter prey for the otter in the study area (Clavero *et al.*, 2004) and amphibians and reptiles are apparently captured in different locations or using different techniques to those used for crayfish consumption. The strong association of these two prey types with insects could be explained by secondary ingestion. In fact Carss & Parkinson (1996) showed that the remains of small fish placed in the gut of larger ones could be identified in otter spraints. However, the same authors also proved that otters predate actively on aquatic insects such as *Dytiscus* beetles. In the study area small animals such as dragonfly nymphs or shrimps were frequent otter prey and were often the only prey types found in spraints (Clavero *et al.*, 2004).

#### REPTILE CONSUMPTION

Little attention has been paid to otter predation on reptiles, mainly due to its almost complete absence in otter diet in central and northern European studies. Ruiz-Olmo (1995) showed that reptiles were not included in the diet in Europe north of 43°N, while Clavero *et al.* (2003) reported a clear increase in reptile

consumption in Mediterranean European locations in relation to temperate ones. This inverse relation between latitude and reptile consumption is a common feature of many carnivore species which occupy a wide latitudinal range (Delibes *et al.*, 1997; Zielinski *et al.*, 1999).

Terrapins have been rarely cited as otter prey (Barrio & Bosch, 1997). In a review of 12 studies of reptile species consumed by the otter Ruiz-Olmo (1995) found a ratio of snakes to terrapins of 177.5:1, suggesting that the hard shell of terrapins would account for their low frequency of occurrence. However, Beja (1996a) reported a ratio snakes to terrapins of 2:1 in southern Portugal, while in this study terrapins were more frequent than snakes in the diet, with a ratio of 1:2.5. The analysis of otter spraints revealed that small terrapins were eaten entirely, since numerous fragments of the bony shell could be found in spraints. That was not the case for larger individuals, which were apparently mutilated by the otter (see Barrio & Bosch, 1997). Remains of large terrapins in spraints included legs, tails, jaws and marginal horny scutes, but not pieces of the bony shell.

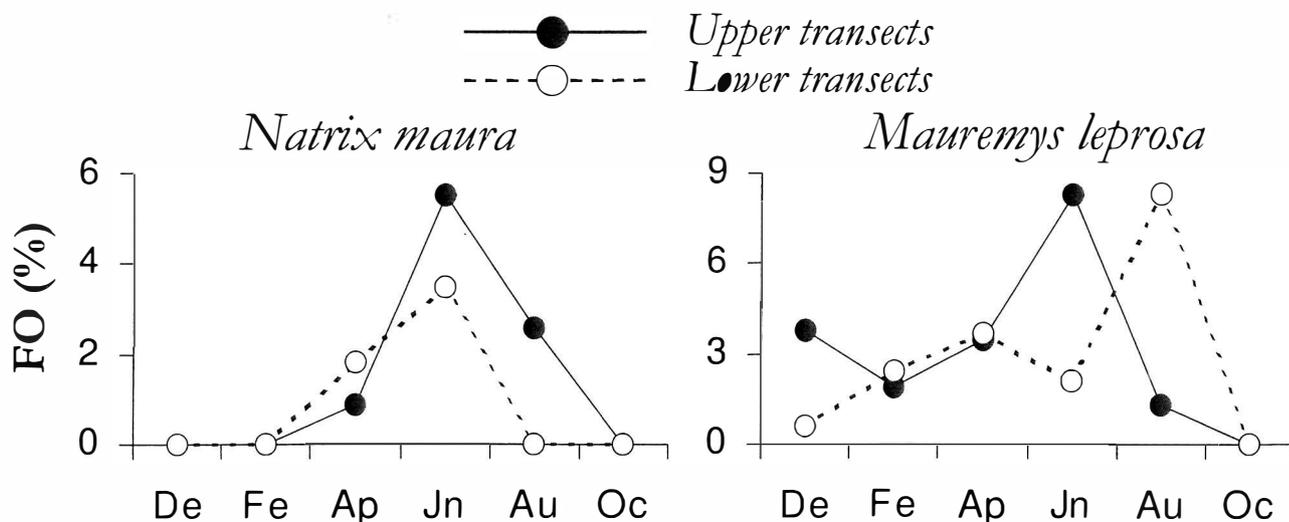


FIG 4. Yearly consumption of viperine snakes (*Natrix maura*) and leprous terrapins (*Mauremys leprosa*) by otters in upper and lower transects of the study area.

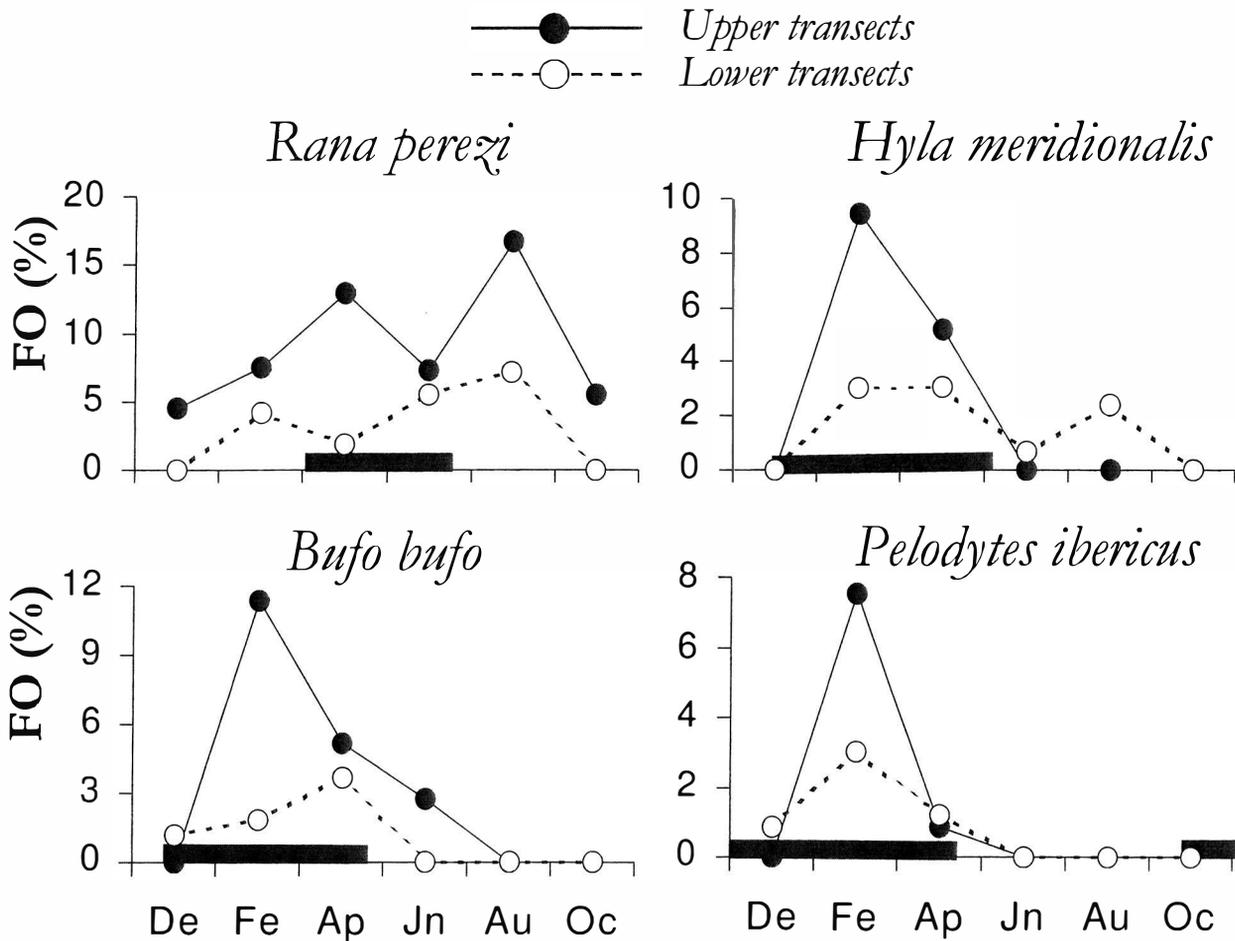


FIG. 5. Yearly consumption of Iberian green frogs (*Rana perezi*), stripeless tree frogs (*Hyla meridionalis*), common toads (*Bufo bufo*) and Iberian parsley frogs (*Pelodytes ibericus*) by otters in upper and lower transects of the study area. The thick lines on the x-axis mark the spawning period in southern Iberian Peninsula following Salvador & García París (2001).

Reptile consumption showed a continuous increase from winter to summer, following the increased activity of leprous terrapins and viperine snakes at higher temperatures (Salvador & Pleguezuelos, 2002). Nevertheless, the different activity periods and habitat use of both species is reflected in their consumption by otters. Due to the mild winter temperatures, terrapins maintain their activity throughout the whole year in southern Iberia, though it shows a clear increase in spring and summer (Andreu & López-Jurado, 1998). This yearly activity pattern would allow otters to predate upon terrapins even in the colder months, as is shown in Fig. 4. On the other hand, viperine snakes hibernate at least from November to March even in southern areas of the Iberian Peninsula. Moreover, snakes are mostly diurnal and spend an important proportion of the time on land in spring and autumn, being nocturnal and largely aquatic in the summer (Braña, 1998). Since south Iberian otters are almost exclusively nocturnal (Beja, 1996b), it is possible that the marked peak of viperine snake consumption in June coincides with the moment when encounters between otters and active snakes are more likely to occur.

AMPHIBIAN CONSUMPTION

Amphibians are important prey for the otter in many European locations, occurring independently of latitude (Jêdrzejewska *et al.*, 2001 and Clavero *et al.*, 2003). However, in most of the diet studies from central and northern Europe, common frogs (*Rana temporaria*) and occasionally common toads are the only amphibian species consumed by the otter (i.e. Weber, 1990; Brzeziński *et al.*, 1993; Sulkava, 1996). In our study area a minimum of six different amphibian species were preyed upon. The Iberian green frog was the most frequent amphibian in the diet, but the proportion of ranids in relation with other amphibians was much lower than in most published studies. The frequency of occurrence of stripeless tree frogs and Iberian parsley frogs was unusually high. Up to seven Iberian parsley frogs were found in a single spraint.

Though some authors proposed that otters do not eat toads due to their venomous skin (Jenkins *et al.*, 1979; Fairley, 1984; De Jongh, 1988), since the first description on otter predation on toads by Lizana & Pérez Mellado (1990) many studies have shown that toads can be common otter prey. Otters avoid the ingestion of the

toads' poisonous substances by skinning them, a behaviour that has been described in Portugal (Beja, 1996a), Spain (Lizana & Pérez Mellado, 1990), Belarus (Sidorovich & Pikulik, 1997) or Finland (Sulkava, 1996). Several common toad skins were observed in the study area during the spraint collection. The ratio of frogs to toads is nevertheless very high in most of the studies, with a clear preponderance of the former (Weber, 1990; Brzeziński *et al.*, 1993; Sidorovich & Pikulik, 1997). However, in our study this ratio was 2.6:1, while Beja (1996a) reported less frequent predation on frogs than on toads, with a ratio of 1:1.2. The occurrence of toads in the diet could have been underestimated in some studies in which amphibians were identified mainly by jaw bones (see discussion in Sulkava, 1996), since skinned toads' skulls are rarely eaten by otters (Lizana & Pérez Mellado, 1990). This problem should be avoided using the ilia bone for specific identifications.

Most of the studies reporting annual patterns on amphibian consumption by otters (Weber, 1990; Brzeziński *et al.*, 1993; Sulkava, 1996) show that the highest occurrence of amphibians occur in late winter and early spring, when common frogs are either hibernating or spawning. This seasonal pattern is related to the habitat use of common frogs, a species which is absent in southern Iberian Peninsula, and that after spawning moves to adjacent woodlands (Weber, 1990). In the study area most amphibian species do not hibernate, but remain active throughout the whole year (Salvador & García París, 2001). However, for most of the species the more intense predation events by otters do occur during spawning periods, in winter and early spring. This is the period when the diversity of amphibians preyed upon by the otter reaches its highest values. The exception to this pattern is the Iberian green frog, with a maximum occurrence in otter diet during the summer that does not overlap with spawning events. In fact, the Iberian green frog is practically the only amphibian consumed by the otter in summer months. This species is strictly aquatic, remaining in streams throughout the year, in contrast with the terrestrial habits of other anurans in the study area and the common frog in other European locations (Salvador & García París, 2001).

In October, with temperatures being still high, there was a sharp decrease in the consumption of all amphibian and reptile species (Fig. 4 and 5). In this period leprous terrapins and Iberian green frogs are still active and thus available for the otter. But at this time the frequency of occurrence of grey mullet, eels and flatfish experienced a pronounced increase. These fishes constitute the bulk of otter diet in the area (Clavero *et al.*, 2004) and are scarcely predated during the summer (Clavero, 2004). It is possible that the autumnal minimum in amphibian and reptile consumption is the result of a switch in otter predation, that during this period, would be centred on estuarine fishes.

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## REFERENCES

- Adrián, M. I. & Delibes, M. (1987). Food habits of the otter (*Lutra lutra*) in two habitats of the Doñana National Park, SW Spain. *Journal of Zoology, London* **12**, 399-406.
- Andreu, A. C. & López-Jurado, L. F. (1998). *Mauremys leprosa* (Schweigger, 1812). In *Reptiles*, 102-108. Salvador, A. (Coord.). Fauna Ibérica, Vol. 10. Madrid: Museo Nacional de Ciencias Naturales.
- Arcá, G. & Prigioni, C. (1987). Food of the otter on the Fiora River (central Italy). *Acta Theriologica* **32**, 134-140.
- Barrio, C. & Bosch, J. (1997). *Mauremys leprosa* como presa de *Lutra lutra*. *Doñana, Acta Vertebrata* **24**, 191-192.
- Beja, P. R. (1996a). An analysis on otter *Lutra lutra* predation on introduced American crayfish *Procambarus clarkii* in Iberian streams. *Journal of Applied Ecology* **33**, 1156-1170.
- Beja, P. R. (1996b). Temporal and spatial patterns of rest-site use by four female otters *Lutra lutra* along the south-west coast of Portugal. *Journal of Zoology, London* **239**, 741-753.
- Beja, P. R. (1997). Predation by marine-feeding otters (*Lutra lutra*) in south-west Portugal in relation to fluctuating food resources. *Journal of Zoology, London* **242**, 503-518.
- Boulenger, G. A. (1897). *Tailless batrachians of Europe*. London: The Ray Society.
- Braña, F. (1998). *Natrix maura* (Linnaeus, 1758). In: *Reptiles*, 440-454. Salvador, A. (Coord.). Fauna Ibérica. Vol. 10. Madrid: Museo Nacional de Ciencias Naturales.
- Brzeziński, M., Jędrzejewski, W. & Jędrzejewska, B. (1993). Diet of otters (*Lutra lutra*) inhabiting small rivers in the Bialowieza National Park, eastern Poland. *Journal of Zoology, London* **230**, 495-501.
- Carss, D. N. (1995). Foraging behaviour and feeding ecology of the otter *Lutra lutra*: a selective review. *Hystrix* **7**, 179-194.
- Carss, D. N. & Parkinson, S. G. (1996). Errors associated with otter *Lutra lutra* faecal analysis. I. Assessing general diet from spraints. *Journal of Zoology, London* **238**, 301-317.
- Clavero, M. (2004). *Ecología y conservación de la nutria y los peces continentales en pequeños arroyos del*

- Campo de Gibraltar*. Huelva: PhD Thesis, Universidad de Huelva.
- Clavero, M., Rebollo, A., Valle, J., Blanco, F., Narváez, M., Delibes, M. & Prenda, J. (2002). Distribución y conservación de la ictiofauna continental en pequeños cursos de agua del Campo de Gibraltar. *Almoraima*, **27**, 335-342.
- Clavero, M., Prenda, J. & Delibes, M. (2003). Trophic diversity of the otter (*Lutra lutra* L.) in temperate and Mediterranean freshwater habitats. *Journal of Biogeography* **30**, 761-769.
- Clavero, M., Prenda, J. & Delibes, M. (2004). The influence of spatial heterogeneity on coastal otters (*Lutra lutra*) prey consumption. *Annales Zoologici Fennici*, **41** 545-549.
- De Jongh, A. W. J. J. (1988). The lack of toad remains in otter *Lutra lutra* spraints. *Lutra* **31**, 79-81.
- Delibes, M., Blázquez, M. C., Rodríguez-Estrella, R. & Zapata, S. C. (1997). Seasonal food habits of bobcats (*Lynx rufus*) in subtropical Baja California Sur, Mexico. *Canadian Journal of Zoology* **74**, 478-483.
- Erlinge, S. (1968). Food studies on captive otters *Lutra lutra* L. *Oikos*. **19**, 259-270.
- Erlinge, S. (1972). Interspecific relations between otter (*Lutra lutra*) and mink (*Mustela vison*) in Sweden. *Oikos* **23**, 327-335.
- Fairley, J. S. (1984). Otters feeding on breeding frogs. *Irish Naturalist* **21**, 372.
- Felix, J. & Montori, A. (1986). Determinación de las especies de anfibios anuros del nordeste ibérico mediante el hueso ilion. *Miscelanea Zoologica* **10**, 239-246.
- Ibarra, P. (1993). *Naturaleza y hombre en el Sur del Campo de Gibraltar: un análisis paisajístico integrado*. Junta de Andalucía, CMA, AMA, Sevilla.
- Jêdrzejewska, B., Sidorovich, V. E., Pikulik, M. M. & Jêdrzejewski, W. (2001). Feeding habits of the otter and the American mink in Bialowieza Primeval Forest (Poland) compared to other Eurasian populations. *Ecography* **24**, 165-180.
- Jenkins, D., Walker, J. G. K. & McCowan, D. (1979). Analyses of otter (*Lutra lutra*) faeces from Deeside, N. E. Scotland. *Journal of Zoology, London* **187**, 235-244.
- Lizana, M. & Pérez Mellado, V. (1990). Depredación por la nutria (*Lutra lutra*) del sapo de la Sierra de Gredos (*Bufo bufo gredosicola*). *Doñana, Acta Vertebrata* **17**, 109-112.
- López-Nieves, P. and Hernando, J. A. (1984). Food habits of the otter in central Sierra Morena (Córdoba, Spain). *Acta Theriologica* **29**, 383-401.
- Mason, C. F. & Macdonald, S. M. (1986). *Otters: ecology and conservation*. Cambridge University Press, Cambridge.
- Pleguezuelos, J. M., Márquez, R. & Lizana, M. (eds.) (2002). *Atlas y libro rojo de los anfibios y reptiles de España*. Dirección General de Conservación de la Naturaleza-Asociación Herpetológica Española, Madrid.
- Ruiz-Olmo, J. (1995). The reptiles in the diet of the otter (*Lutra lutra* L., Carnivora, Mammalia) in Europe. In: *Scientia Herpetologica*. Llorente, G., Santos, X. & Carretero, M. A. (eds.). Asociación Herpetologica Española, Barcelona.
- Salvador, A. & García París, M. (2001). *Anfibios españoles*. Canseco Editores, Talavera de la Reina, Spain.
- Salvador, A. & Pleguezuelos, J. M. (2002). *Reptiles españoles*. Canseco Editores, Talavera de la Reina, Spain.
- Sidorovich, V. E. & Pikulik, M. M. (1997). Toads *Bufo* spp. in the diets of mustelid predators in Belarus. *Acta Theriologica* **42**, 105-108.
- Simões-Graça, M. A. & Ferrand de Almeida, F. X. (1983). Contribuição para o conhecimento da lontra (*Lutra lutra* L.) num sector da bacia do Rio Mondego. *Cienc. Biol. Ecol. Syst.* **5**, 33-42.
- Sulkava, R. (1996). Diet of otters *Lutra lutra* in central Finland. *Acta Theriologica* **41**, 395-408.
- Weber, J. M. (1990). Seasonal exploitation of amphibians by otters (*Lutra lutra*) in north-east Scotland. *Journal of Zoology, London* **220**, 641-651.
- Zar, J. H. (1984). *Biostatistical analysis, 2<sup>nd</sup> edn*. Prentice Hall, Englewood Cliffs.
- Zielinski, W. J., Duncan, N. P., Farmer, E. C., Truex, R. L., Clevenger, A. P., & Barret, R. H. (1999). Diet of fishers (*Martes pennanti*) at the southernmost extent of their range. *Journal of Mammalogy* **80**, 961-971.

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## SHORT NOTES

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**ENDOPARASITES INFECTING TWO SPECIES OF WHIPTAIL LIZARD (*CNEMIDOPHORUS ABAETENSIS* AND *C. OCELLIFER*; TEIIDAE) IN A 'RESTINGA' HABITAT OF NORTH-EASTERN BRAZIL**

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We analysed the endoparasite fauna associated with two species of whiptail lizard (*Cnemidophorus abaetensis* and *C. ocellifer*) from north-eastern Brazil. Overall parasite prevalence was relatively low for both species (18.2% in *C. abaetensis* and 12.5% in *C. ocellifer*). Four parasite species were recorded: the pentastomid *Raillietiella* aff. *furcocerca* and the nematodes *Physaloptera lutzi*, *P. retusa* and *Hexametra boddaertii*. We compared our results with those of previous similar studies on other whiptails.

*Key words:* helminths, parasites, squamates

Considering the great richness of the Brazilian squamate fauna, very little is known of the associated endoparasite faunas of most species. Most available information on endoparasite faunas of Brazilian squamates pertains to nematodes, with data on relatively few host species (e.g. Baker, 1987; Vicente *et al.*, 1993; Rocha *et al.*, 2000b). Studies considering helminth community parameters such as prevalence, abundance and infection intensity (*sensu* Bush *et al.*, 1997) for Brazilian squamates are even scarcer and deal only with lizards, with most of them being fairly recent (e.g. Van Sluys *et al.*, 1997; Ribas *et al.*, 1995; 1998; Rocha, 1995; Vrcibradic *et al.*, 1999, 2000, 2002 a,b; Rocha *et al.*, 2003; Rocha & Vrcibradic, 2003).

The whiptail lizards (cnemidophorines *sensu* Reeder *et al.*, 2002) are the most widespread members of the family Teiidae, occurring throughout most of the United States, Mexico and Central and South America, including the Caribbean region (Wright, 1993; Reeder *et al.*, 2002). Information on the endoparasite faunas of whiptail lizards exist for several Nearctic (Telford, 1970; Dyer, 1971; Benes, 1985; Lyon, 1986; McAllister *et al.*, 1986; 1991a,b; Goldberg & Bursey, 1989; 1990; 2003; McAllister, 1990a,b,c,d; 1992; Telford & Bursey, 2003) and a few Neotropical taxa (Specian & Whittaker,

1980; Ribas *et al.*, 1995; 1998; Vrcibradic *et al.*, 2000; Menezes *et al.*, 2004). In this study we survey the endoparasite faunas of two sympatric species of whiptail lizards from Brazil, *Cnemidophorus abaetensis* Dias, Rocha & Vrcibradic, 2002 and *Cnemidophorus ocellifer* (Spix, 1824). *Cnemidophorus abaetensis* is a recently described species whose geographic distribution is apparently restricted to the northern coast of Bahia state (Dias *et al.*, 2002), whereas *C. ocellifer* is widespread in South America south of the Amazonian region, from north-eastern and central Brazil to Paraguay, Bolivia and northern Argentina (Vanzolini *et al.*, 1980; Cei, 1993). So far, nothing has been published about the endoparasites associated with these two species [in the study of Ribas *et al.*, (1995) regarding nematodes of *C. ocellifer*, the species under treatment is actually *C. littoralis* Rocha, Araújo, Vrcibradic & Costa, 2000, which had not yet been formally described at the time (see Rocha *et al.*, 2000a)].

A total of 73 lizards (33 *C. abaetensis* and 40 *C. ocellifer*) were collected by the first author with the aid of elastic rubber bands at the coastal sand-dune ('restinga') habitat of Dunas do Abaeté (12° 57' S, 38° 22' W), Salvador municipality, Bahia state, north-eastern Brazil, from March to May 2000. Whenever a lizard was sighted, the band was stretched and released so that the knot would hit the animal and stun it (whereupon it was captured). Immediately upon collection, the animals were humanely euthanased and their snout-vent length measured with a calliper (to the nearest 0.1 mm), before fixation with 10% formalin. In the laboratory, lizards were dissected and their digestive tract was removed, opened and examined for the presence of parasites. The lizards' body cavities and lungs were also examined. All parasites found were mounted on temporary slides for identification (nematodes were cleared in phenol and pentastomids were treated with Hoyer solution). The proportion of infected individuals was compared between species and between sexes for each host species using the Z-test for proportions (Zar, 1999). Ecological-parasitological terminology used throughout the text follow Bush *et al.* (1997). Our host samples can be considered as relatively homogeneous, since the lizards were all collected within a relatively short time period (see Janovy & Kutish, 1988). All lizards surveyed in the present study are deposited at the herpetological collection of the Museu Nacional, Rio de Janeiro (*C. abaetensis*: MNRJ 8617-49; *C. ocellifer*: MNRJ 10984-1 1023). Voucher specimens of parasites found in the present study are deposited at the parasite collection of the Instituto Oswaldo Cruz (CHIOC 34834-8), in Rio de Janeiro, Brazil, at the zoological collection of the Universidade Regional do Cariri (LZ-URCA 0036-8), in Crato, Brazil, and at the U. S. National Parasite Collection (USNPC 92570-1).

Six (18.2%) of the 33 *C. abaetensis* (mean SVL=54.8±10.6 mm; range 37.4-70.4 mm) and five (12.5%) of the 40 *C. ocellifer* (mean SVL=50.6±5.1 mm; range 37.9-64.5 mm) harboured endoparasites.

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There was no significant difference in overall prevalence between the two lizard species ( $Z=0.675$ ,  $P=0.25$ ). Overall, four parasite species were identified from the lizards: one pentastomid [*Raillietiella* aff. *furcocerca* (Diesing, 1835)] and three nematodes [*Physaloptera lutzi* Cristófaró, Guimarães and Rodrigues, 1976 and *P. retusa* (Rudolphi, 1819) (Physalopteridae), and *Hexametra boddaertii* (Baird, 1860) (Ascarididae)]. The latter species was found only as a third stage larvae. Two specimens of *Physaloptera* could not be identified to species due to their immature stage and consequent lack of taxonomically diagnostic structures. Parasites were all site-specific: pentastomids were found in lungs, *Physaloptera* spp. were found in the stomach and *Hexametra* was found in the coelom (usually encysted in peritoneal membranes). Epidemiological data for each parasite species in either host is presented in Table 1. No individual lizard of either species harboured more than one parasite species.

The proportion of infected individuals did not differ between males (2/15) and females (4/18) of *C. abaetensis* ( $Z=0.742$ ,  $P=0.229$ ) nor between males (4/20) and females (1/20) of *C. ocellifer* ( $Z=1.434$ ,  $P=0.076$ ).

All parasites recorded in this study represent new host records. *Physaloptera retusa* and *P. lutzi* have been previously reported from a number of other lizard species in different families (e.g. Baker, 1987; Vicente *et al.*, 1993; Van Sluys *et al.*, 1997; Ribas *et al.*, 1995; 1998; Rocha, 1995; Roca, 1997; Rocha *et al.*, 2003; Menezes *et al.*, in press). *Hexametra boddaertii*, a parasite of snakes (Sprent, 1978), has been previously found as larvae in other Brazilian lizards (Vrcibradic *et al.*, 1999; 2000), and apparently uses lizards as paratenic hosts. The pentastomid *Raillietiella furcocerca* is currently known from several neotropical snake species, albeit not from lizards (Rego, 1983; Ali *et al.*, 1984); the specimens reported in the present study may actually represent a new species closely related to *R. furcocerca* (W. O. Almeida, pers. comm.).

The two *Cnemidophorus* species at the Dunas de Abaeté presented low prevalences and intensities of infection by endoparasites. Populations of the congener *C. littoralis* studied in two restinga areas in south-eastern

Brazil (Barra de Maricá and Jurubatiba) showed much higher overall prevalences (86% and 77%, respectively) and mean infection intensities (13.1 and 9.2, respectively), even though surveys were restricted to nematodes (Ribas *et al.*, 1995; Rocha *et al.*, 2000b; Vrcibradic *et al.*, 2000). On the other hand, no nematodes were found (other parasite types were not surveyed) in a sample of 15 *C. nativo* Rocha, Bergallo & Peccinini-Seale, 1997 from another south-eastern Brazilian restinga studied by Van Sluys *et al.* (1997). This compares to another population of the same species from a different restinga that had an overall endoparasite prevalence of 35% (Menezes *et al.*, 2004), which is intermediate between the low values reported for *C. abaetensis* and *C. ocellifer* in the present study and the high ones reported for *C. littoralis* (see above). Such differences in infection prevalences and intensities among species/populations of those closely related taxa (all of which belong to the so-called "ocellifer" complex; see Dias *et al.*, 2002) may reflect differences among localities in the abundance of nematodes and/or their intermediate hosts. Populations of Brazilian skinks of the genus *Mabuya* from different restinga areas are known to vary widely in nematode infection parameters such as overall prevalence and intensity of infection (see Rocha & Vrcibradic, 2003).

Moreover, none of the four parasite species infecting the two *Cnemidophorus* occurred with sufficient prevalence (i.e.  $\geq 10\%$ ) to be considered as component species (see Bush *et al.*, 1990). Studies on several North American whiptail species/populations have reported overall helminth prevalences ranging from 8% to 50% (usually  $\geq 25\%$ ), with the number of component species ranging from zero to four (most commonly one) (Telford, 1970; Benes, 1985; Lyon, 1986; Goldberg & Bursey, 1989; 1990; McAllister, 1990a,b,c,d; 1992; McAllister *et al.*, 1991a,b; Telford & Bursey, 2003). This, coupled with the aforementioned helminth prevalences reported for other Brazilian whiptails (*C. littoralis* and *C. nativo*), indicates that the two *Cnemidophorus* populations studied here appear to present low infection rates for whiptails in general.

The low prevalences of parasites in the two whiptail species do not allow the identification of possible differ-

TABLE 1. Data on the prevalences (in absolute numbers and percentages) and mean infection intensities (with range in parentheses) for each endoparasite species infecting *Cnemidophorus abaetensis* and *C. ocellifer* at Dunas do Abaeté, Bahia, Brazil. The letter "I" designates parasite taxa that were found only in immature stages.

	<i>C. abaetensis</i> (n=33)		<i>C. ocellifer</i> (n=40)	
	Prevalence(%)	Intensity	Prevalence(%)	Intensity
Pentastomida				
<i>Raillietiella</i> aff. <i>furcocerca</i>	2(6.0%)	4.5(3-6)	2(2.5%)	1
Nematoda				
<i>Hexametra boddaertii</i> (I)			3(7.5%)	6.7(1-12)
<i>Physaloptera lutzi</i>	2(6.0%)	2.5(1-4)		
<i>Physaloptera retusa</i>	1(3.0%)	1		
<i>Physaloptera</i> sp. (I)	1(3.0%)	1	1(2.5%)	1

ences in endoparasite community patterns between them. There may be a trend for *C. ocellifer* to be less susceptible to infections by *Physaloptera* spp. and more susceptible to acquire *Hexametra* than *C. abaetensis*, but the low prevalences obscure any potential pattern. Also, there does not appear to be a clear tendency, in either species, for one of the sexes to be more prone to infections than the other. Since the present study deals with two congeneric (and thus closely related) species, we cannot speculate if the low infection rates and species-poor endoparasite communities observed reflect a general scarcity of lizard parasites in the study area or a lower susceptibility to parasitism in those whiptails compared to other sympatric lizards. The already mentioned high prevalences of nematode parasites in populations of the closely related congener *C. littoralis* from other resting areas (Ribas *et al.*, 1995; Vrcibradic *et al.*, 2000) seems to suggest the former. Since many of the parasites (such as physalopterids) are acquired via ingestion of infected intermediary hosts (mostly arthropods), possible dietary differences among whiptail species could result in differential parasite prevalences. This does not seem to be the case, however, since the diets of the two species studied here (Dias, 2002) do not seem to differ much from that of *C. littoralis* (Teixeira-Filho *et al.*, 2003) or *C. nativo* (Menezes, 2003), with all four species feeding predominantly on termites but also consuming a wide variety of other arthropods. Endoparasite surveys of other lizard species occurring sympatrically with the two whiptails at Dunas do Abaeté might help to settle the matter.

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#### REFERENCES

- Ali, J. H., Riley, J. & Self, J. T. (1984). A revision of the taxonomy of pentastomid parasites (genus *Raillietiella* Sambon, 1910) from American snakes and amphisbenians. *Systematic Parasitology* **6**, 87-97.
- Baker, M. R. (1987). Synopsis of the nematoda parasitic in amphibians and reptiles. *Memoirs of the University of Newfoundland. Occasional Papers in Biology* **11**, 1-325.
- Benes, E. S. (1985). Helminth parasitism in some central Arizona lizards. *The Southwestern Naturalist* **30**, 467-473.
- Bush, A. O., Aho, J. M. & Kennedy, C. R. (1990). Ecological versus phylogenetic determinants of helminth parasite community richness. *Evolutionary Ecology* **4**, 1-20.
- Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. (1997). Parasitology meets ecology in its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575-583.
- Cei, J. M. (1993). Reptiles del Noroeste, Nordeste y Este de la Argentina. Herpetofauna de las selvas subtropicales, puna y pampas. *Monografía del Museo Regionale di Scienze Naturali, Torino* **14**, 1-949.
- Dias, E. J. R. (2002). Ecologia de duas espécies simpátricas de lagartos (*Cnemidophorus* sp. n. e *C. ocellifer* - Teiidae) das Dunas de Abaeté, Salvador, Bahia, Brasil. Unpubl. Master's dissertation, Universidade do Estado do Rio de Janeiro. 145 p.
- Dias, E. J. R., Rocha, C. F. D. & Vrcibradic, D. (2002). New *Cnemidophorus* (Squamata: Teiidae) from Bahia state, north-eastern Brazil. *Copeia* **2002**, 1070-1077.
- Dyer, W. G. (1971). Some helminths of the six-lined lizard, *Cnemidophorus sexlineatus*, in South Dakota. *Proceedings of the Helminthological Society of Washington* **38**, 256.
- Goldberg, S. R. & Bursey, C. R. (1989). Helminths of the giant spotted whiptail, *Cnemidophorus burti stictogrammus* (Sauria: Teiidae). *Proceedings of the Helminthological Society of Washington* **56**, 86-87.
- Goldberg, S. R. & Bursey, C. R. (1990). Helminths of the Arizona little striped whiptail, *Cnemidophorus inornatus arizonae*, and the desert grassland whiptail, *Cnemidophorus uniparens* (Sauria: Teiidae), from southeastern Arizona. *Journal of the Helminthological Society of Washington* **57**, 83-86.
- Goldberg, S. R. & Bursey, C. R. (2003). *Cnemidophorus hyperythrus* (Orange-throated Whiptail). Endoparasites. *Herpetological Review* **34**, 142.
- Janovy, J. & Kutish, G. W. (1988). A model of encounters between host and parasite populations. *Journal of Theoretical Biology* **134**, 391-401.
- Lyon, R. E. (1986). Helminth parasites of six lizard species from southern Idaho. *Proceedings of the Helminthological Society of Washington* **53**, 291-293.
- McAllister, C. T. (1990a). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. I. The Colorado checkered whiptail (*Cnemidophorus tesselatus*). *Journal of Wildlife Diseases* **26**, 139-142.
- McAllister, C. T. (1990b). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. II. The New Mexico whiptail (*Cnemidophorus neomexicanus*). *Journal of Wildlife Diseases* **26**, 403-406.
- McAllister, C. T. (1990c). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. III. The Chihuahuan spotted whiptail (*Cnemidophorus exsanguis*). *Journal of Wildlife Diseases* **26**, 544-546.

- McAllister, C. T. (1990d). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. IV. The Texas spotted whiptail (*Cnemidophorus gularis*). *Texas Journal of Science* **42**, 381-388.
- McAllister, C. T. (1992). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. VIII. The Gila spotted whiptail (*Cnemidophorus flagellicaudus*), Sonoran spotted whiptail (*Cnemidophorus sonorae*), and Plateau striped whiptail (*Cnemidophorus velox*). *Texas Journal of Science* **44**, 233-239.
- McAllister, C. T., Cordes, J. E. & Walker, J. M. (1991a). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America .VI. The gray checkered whiptail (*Cnemidophorus dixonii*). *Texas Journal of Science* **43**, 309-314.
- McAllister, C. T., Trauth, S. E. & Conn, D. B. (1991b). Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. VII. The 6-lined racerunner (*Cnemidophorus sexlineatus*). *Texas Journal of Science* **43**, 391-397.
- McAllister, C. T., Trauth, S. E. & Ubelaker J. E. (1986). Nematode parasites of the parthenogenetic whiptail lizard, *Cnemidophorus laredoensis* (Sauria, Teiidae) from south Texas. *Proceedings of the Helminthological Society of Washington* **53**, 138-139.
- Menezes, V. A. (2003). Ecologia do lagarto partenogenético *Cnemidophorus nativo* (Teiidae) na restinga de Guaratiba, Prado, BA. Unpubl. Master's dissertation, Universidade do Estado do Rio de Janeiro. 108 p.
- Menezes, V. A., Vrcibradic, D., Vicente, J. J., Dutra, G. F. & Rocha, C. F. D. (in press). Helminths infecting the parthenogenetic whiptail lizard *Cnemidophorus nativo* in a restinga habitat of Bahia State, Brazil. *Journal of Helminthology* (in press).
- Reeder, T. W., Cole, C. J. & Dessauer, H. C. (2002). Phylogenetic relationships of whiptail lizards of the genus *Cnemidophorus* (Squamata: Teiidae): a test of monophyly, reevaluation of karyotypic evolution, and review of hybrid origins. *American Museum Novitates* **3365**, 1-61.
- Rego, A. A. (1983). Pentastomídeos de répteis do Brasil: Revisão dos Cephalobaenidae. *Memórias do Instituto Oswaldo Cruz* **78**, 399-411.
- Ribas, S. C., Rocha, C. F. D., Teixeira-Filho, P. F. & Vicente, J. J. (1995). Helminths (Nematoda) of the lizard *Cnemidophorus ocellifer* (Sauria: Teiidae): assessing the effect of rainfall, body size and sex in the nematode infection rates. *Ciência e Cultura* **47**, 88-91.
- Ribas, S. C., Rocha, C. F. D., Teixeira-Filho, P. F. & Vicente, J. J. (1998). Nematode infection in two sympatric lizards (*Tropidurus torquatus* and *Ameiva ameiva*) with different foraging tactics. *Amphibia-Reptilia* **19**, 323-330.
- Roca, V. (1997). Natural history notes. *Tropidurus melanopleurus*. Parasites. *Herpetological Review* **28**, 204.
- Rocha, C. F. D. (1995). Nematode parasites of the Brazilian sand lizard, *Liolaemus lutzae*. *Amphibia-Reptilia* **16**, 412-415.
- Rocha, C. F. D. & Vrcibradic, D. (2003). Nematode assemblages of some of some insular and continental lizard hosts of the genus *Mabuya* Fitzinger (Reptilia, Scincidae) along the eastern Brazilian coast. *Revista Brasileira de Zoologia* **20**, 755-759.
- Rocha, C. F. D., Araújo, A. F. B., Vrcibradic, D. & Costa, E. M. M. (2000a). New *Cnemidophorus* (Squamata: Teiidae) from coastal Rio de Janeiro state, southeastern Brazil. *Copeia* **2000**, 501-509.
- Rocha, C. F. D., Vrcibradic, D. & Araújo, A. F. B. (2000b). Ecofisiologia de répteis de restingas brasileiras. In *Ecologia de Restingas e Lagoas costeiras*, 117-149. Esteves F. A. and Lacerda. L. D. (Eds). Macaé: NUPEM/UFRJ.
- Rocha, C. F. D., Vrcibradic, D., Vicente, J. J. & Cunha-Barros, M. (2003). Helminths infecting *Mabuya dorsivittata* (Lacertilia, scincidae) from a high-altitude habitat in Itatiaia National Park, Rio de Janeiro State, southeastern Brazil. *Brazilian Journal of Biology* **63**, 129-132.
- Specian, R. D. & Whittaker, F. H. (1980). A survey of the helminth fauna of *Cnemidophorus murinus* from the island of Curaçao. *Proceedings of the Helminthological Society of Washington* **47**, 275-276.
- Sprent, J. F. A. (1978). Ascaridoid nematodes of amphibians and reptiles: *Polydelphis*, *Travassosascaris* n.g. and *Hexametra*. *Journal of Helminthology* **52**, 355-384.
- Teixeira-Filho, P. F. & Rocha, C. F. D. (2003). *Relative feeding specialization may depress ontogenetic, seasonal and sexual variations in diet: the endemic lizard Cnemidophorus littoralis (Teiidae)*. *Brazilian Journal of Biology* **63**, 321-328.
- Telford, S. R. (1970). A comparative study of endoparasitism among some southern California lizard populations. *American Midland Naturalist* **83**, 516-554.
- Telford, S. R. & Bursey, C. R. (2003). Comparative parasitology of squamate reptiles endemic to scrub and sandhill communities of north-central Florida, U.S.A. *Comparative Parasitology* **70**, 172-181.
- Van Sluys, M., Rocha, C. F. D., Bergallo, H. G., Vrcibradic, D. & Ribas, S. C. (1997). Nematode infection in three sympatric lizards in an isolated fragment of restinga habitat in southeastern Brazil. *Amphibia-Reptilia* **18**, 442-446.
- Vanzolini, P. E., Ramos-Costa, A. M. M. & Vitt, L. J. (1980). *Répteis das Caatingas*. Rio de Janeiro: Academia Brasileira de Ciências. 161 p.
- Vicente, J. J., Rodrigues, H. O., Gomes, D. C. & Pinto, R. M. (1993). Nematóides do Brasil. Parte III: Nematóides de Répteis. *Revista Brasileira de Zoologia* **10**, 19-168.
- Vrcibradic, D., Rocha, C. F. D., Ribas, S. C. & Vicente, J. J. (1999). Nematodes infecting the skink *Mabuya frenata* in Valinhos, São Paulo State, southeastern Brazil. *Amphibia-Reptilia*, **20**, 333-339.
- Vrcibradic, D., Cunha-Barros, M., Vicente, J. J., Galdino, C. A. B., Hatano, F. H., Van Sluys, M. & Rocha, C. F. D. (2000). Nematode infection patterns in four

- sympatric lizards from a restinga habitat (Jurubatiba) in Rio de Janeiro state, southeastern Brazil. *Amphibia-Reptilia* **21**, 307-316.
- Vrcibradic, D., Rocha, C. F. D., Bursey, C. R. & Vicente, J. J. (2002a). Helminths infecting *Mabuya agilis* (Lacertilia, Scincidae) in a "restinga" habitat (Grumari) of Rio de Janeiro, Brazil. *Amphibia-Reptilia* **23**, 109-114.
- Vrcibradic, D., Rocha, C. F. D., Bursey, C. R. & Vicente, J. J. (2002b). Helminth communities of two sympatric skinks (*Mabuya agilis* and *Mabuya macrorhyncha*) from two "restinga" habitats in southeastern Brazil. *Journal of Helminthology* **76**, 355-361.
- Wright, J. W. (1993). Evolution of the lizards of the genus *Cnemidophorus*. In *Biology of Whiptail Lizards (genus Cnemidophorus)*, 27-81. Wright, J. W. and Vitt, L. J. (Eds), Oklahoma: Oklahoma Museum of Natural History.
- Zar, J. H. (1999). *Biostatistical Analysis*. 4<sup>th</sup> ed. Upper Saddle River: Prentice-Hall.

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## INFLUENCE OF FLUCTUATING INCUBATION TEMPERATURES ON HATCHLING TRAITS IN A CHINESE SKINK, *EUMECES CHINENSIS*

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Effects of fluctuating temperatures on reptilian eggs and hatchlings are poorly known. We carried out a fluctuating-temperature incubation experiment with two treatments ( $27 \pm 3$  °C vs  $27 \pm 7$  °C) to determine the effects of temperature and clutch of origin on incubation duration and the morphology and locomotor performance of hatchlings in a skink, *Eumeces chinensis*. The variance of fluctuating temperatures had no influences on incubation duration, hatching success or hatchling traits. Incubation temperature accounted for over 10% of the total variation in incubation duration and hatchling traits, whereas clutch effects accounted for much more. The discrepancy between these results and those from previous studies may be due to interspecific differences in embryonic response to thermal variance.

*Key words:* egg incubation, maternal effect, temperature

Most embryonic development occurs outside the mother's body in oviparous reptiles, and incubation environments thus can influence egg survival rates and the phenotypic traits of hatchlings (Deeming & Ferguson, 1991). Temperature is one of the most important such environmental factors, and can profoundly affect incubation duration, hatching success, and hatchling traits such as morphology, behaviour, growth and gender (Deeming & Ferguson, 1991; Packard & Packard, 1988; Burger, 1990; Janzen & Paukstis, 1991). Constant-temperature incubation experiments in the laboratory have been conducted on many reptile species. In general, eggs incubated at moderate temperatures produce relative large hatchlings that perform better than those from low or high temperatures (e.g. Packard & Packard, 1988; Van Damme *et al.*, 1992; Phillips & Packard, 1994; Ji & Du, 2001; Ji & Zhang, 2001; Du & Ji, 2003; Du *et al.*, 2003).

In natural nests of reptiles, however, temperatures fluctuate considerably on a daily and seasonal basis (Shine *et al.*, 1996). Therefore, constant-temperature incubation may be biological unrealistic. More recently, some investigators have conducted incubation experiments either in the laboratory or in natural nests to

determine the effect of fluctuating temperatures on incubating eggs and resultant hatchlings (e.g. Shine *et al.*, 1996, 1997; Ashmore & Janzen, 2003; Shine, 2004). These studies suggest that both the variance and the mean of incubation temperatures significantly affect developmental rates and hatchling traits. Nonetheless, few such studies have been conducted and thus, we do not know the generality of any influence of fluctuating temperatures on phenotypic traits of hatchling reptiles. In the current study, we conducted a fluctuating-temperature incubation experiment to determine the effects of thermal regimes on incubation duration, hatchling morphology and locomotor performance in a Chinese skink, *Eumeces chinensis*.

*Eumeces chinensis* is a small (in our study, snout-vent length: 100.5-134.0 mm), diurnal, oviparous skink widely distributed in the central and southern provinces of China (Zhao & Adler, 1993). In May 2003, we captured four gravid females by hand from Quzhou, Zhejiang, eastern China. The animals were housed in a terrarium (110 × 90 × 50 cm) that had been filled with sand and grass to mimic the natural habitat where the lizards were captured. A 60W light bulb was suspended 15 cm above the terrarium floor as a supplementary heating source from 0700-1700 hr. Food (larvae of *Tenebrio molitor*) and water (containing mixed vitamins and minerals) were provided *ad libitum*. Each female constructed a nest individually under the grass where she later deposited a clutch of eggs. The terrarium was checked three times per day for the presence of laid eggs. All eggs were weighed immediately to the nearest mg to minimize any change in egg mass due to water exchange between eggs and their surroundings.

Clutch sizes of the four females were 8-13 eggs; and a total of 37 eggs were viable. Each clutch was split into two treatments. The eggs were incubated at  $27 \pm 3$  °C and  $27 \pm 7$  °C, respectively. The temperatures in the incubators were programmed to fluctuate over 24 hr. For  $27 \pm 3$  °C treatment, temperatures increased or decreased 1 °C every two hours; for  $27 \pm 7$  °C, temperatures increased or decreased about 1 °C per hour. Temperatures inside the containers were recorded every hour by TINYTALK dataloggers (Gemini Pty, Australia). Prior to being transferred into incubators, eggs were placed in containers filled with moist vermiculite substrates (water potential of -12 kPa; Du *et al.*, 2003). We weighed all containers daily and added water to compensate for evaporative losses and water absorbed by eggs throughout the incubation period, such that the water potential of the substrate remained constant.

After the first hatchling pipped its eggshell, containers were checked at least five times each day. Each hatchling was weighed and its locomotor performance was tested immediately after it had hatched. Because locomotor performance is highly sensitive to body temperature in reptiles, we performed trials after placing hatchlings in an incubator at 30 °C for 30 min prior to testing (Du *et al.*, 2003). We assessed locomotor capac-

ity by chasing the lizards along a 1.5 m racetrack with a paintbrush. The locomotor performance of each lizard was recorded with a Panasonic NV-MX3 digital video camera. Videotapes were then examined for sprint speed in the fastest 150 mm interval and maximum distance in a dash.

After the locomotor tests, we euthanized these hatchlings, and took the following measurements: SVL, tail length, head size (length and width), and limb length (including toe length). Subsequently, the hatchlings were dissected into carcass and residual yolk. The two components were oven-dried to constant mass at 65 °C, and weighed.

Hatching success (80.0%, 12/15) of eggs incubated at 27±3 °C was similar to that (86.4%, 19/22) of eggs kept at 27±7 °C (*G*-test, *G*=0.05, *df*=1, *P*>0.05). We analyzed data from these eggs using mixed model multivariate analysis of covariance where temperature was a fixed factor, clutch of origin was a random factor and egg mass was the covariate. These analyses indicated that incubation temperature did not significantly affect incubation duration or hatchling traits (Table 1). Due to low sample size, we conducted a further analysis that regarded each hatchling as an independent sample to test the effects of temperature on hatchling traits, acknowledging that this may not be statistically appropriate. The results also showed that the hatchling traits were not affected by the temperature regimes. To identify the relative magnitude of effects due to temperature *versus* clutch effects, we calculated the percentage variation in hatchling traits due to clutch, temperature and their interaction from the sums of squares in two-way ANCOVAs where egg mass or hatchling SVL was used as the covariate. Temperature effects only accounted for a small amount of the overall variation in incubation duration and hatchling traits,

with no more than 10 % in most traits. In contrast, clutch (maternal) effects accounted for most of the variation in incubation length and hatchling traits (Table 2).

Our study suggests that thermal variation during incubation does not influence hatchling traits in *E. chinensis*. Given that the air temperatures of the locality where the skinks were captured average 25.0 °C and 29.1 °C during June and July when the natural incubations mostly occur (Quzhou Bureau of Meteorology), we believe that the mean temperatures experienced by eggs in natural nests may largely fall in the range of 24~30 °C. Over this range, mean incubation temperatures do not affect hatching traits in this species (Ji & Zhang, 2001). Taken together, these results suggest that the nest temperature exerts relatively little effect on hatchling traits in *E. chinensis*. Instead, most variation in hatchling traits stems from genetic sources and/or maternal effects (Table 2).

Empirical evidence for the influence of fluctuating temperatures on reptile eggs and resultant hatchlings is limited to studies on relatively few species, and such effects are far from universal (Overall, 1994; Shine *et al.*, 1997; Doody, 1999; Andrews *et al.*, 2000; Ashmore & Janzen, 2003). The incubation duration of *E. chinensis* is independent of diel thermal fluctuation, at least over the range of conditions in our study. This result is consistent with that from *Sceloporus undulatus* (Andrews *et al.*, 2000), but not those from other species, in which incubation duration increased (Shine *et al.*, 1997; Ashmore & Janzen, 2003) or decreased (Overall, 1994) with increasing variation in the fluctuating temperatures. Fluctuating temperatures had significant effects on hatchling size and/or locomotor performance in most species of reptiles that have been studied in this respect (Shine, *et al.*, 1997; Doody, 1999; Andrews *et al.*, 2000; Ashmore & Janzen, 2003; Shine, 2004). On the con-

TABLE 1. ANCOVA results of temperature effects on hatchling traits of *Eumeces chinensis*. ANCOVA with temperature as a fixed factor and clutch of origin as a random factor (Temperature effect 1), and ANCOVA with individual hatchlings as independent samples (Temperature effect 2), were conducted respectively. Egg mass was used as the covariate in analyses of incubation duration, snout-vent length, body mass, carcass and residual yolk. Hatchling SVL was used as the covariate in tail length, head size, limb length and locomotor performance.

	Temperature effect 1	Temperature effect 2
Incubation length	$F_{1,3} = 0.98, P = 0.37$	$F_{1,28} = 0.33, P = 0.57$
Snout-vent length	$F_{1,3} = 0.12, P = 0.76$	$F_{1,28} = 0.10, P = 0.75$
Wet body mass	$F_{1,3} = 0.65, P = 0.48$	$F_{1,28} = 1.84, P = 0.19$
Dry body mass	$F_{1,3} < 0.0001, P = 0.99$	$F_{1,28} < 0.0001, P = 0.99$
Carcass	$F_{1,3} = 0.05, P = 0.84$	$F_{1,28} = 0.07, P = 0.79$
Residual yolk	$F_{1,3} = 0.12, P = 0.76$	$F_{1,28} = 0.15, P = 0.70$
Tail length	$F_{1,3} = 0.008, P = 0.94$	$F_{1,28} = 0.02, P = 0.88$
Head length	$F_{1,3} = 0.22, P = 0.69$	$F_{1,28} = 0.17, P = 0.68$
Head width	$F_{1,3} = 1.60, P = 0.33$	$F_{1,28} = 0.70, P = 0.41$
Forelimb length	$F_{1,3} = 2.01, P = 0.18$	$F_{1,28} = 1.67, P = 0.21$
Hindlimb length	$F_{1,3} = 0.16, P = 0.75$	$F_{1,28} = 0.07, P = 0.79$
Maximum distance	$F_{1,3} = 0.30, P = 0.64$	$F_{1,28} = 0.23, P = 0.63$
Sprint speed	$F_{1,3} = 1.64, P = 0.22$	$F_{1,28} = 0.004, P = 0.95$

TABLE 2. Percentage variation in hatchling traits of *Eumeces chinensis* due to clutch, temperature and interaction respectively. Data were calculated from the sums of squares in two-way ANCOVA. Egg mass was used as the covariate in analyses of incubation duration, snout-vent length, body mass, carcass and residual yolk. Hatchling SVL was used as the covariate in tail length, head size, limb length and locomotor performance.

	Clutch (%)	Temp. (%)	Interaction (%)
Incubation length	96.07	3.32	0.61
Snout-vent length	71.75	0.30	27.95
Wet body mass	69.71	0.01	30.28
Dry body mass	83.02	5.28	11.70
Carcass	81.63	1.83	16.54
Residual yolk	73.52	11.07	15.41
Tail length	62.00	2.39	35.61
Head length	48.55	5.47	45.98
Head width	69.57	1.24	29.19
Forelimb length	65.42	21.30	13.28
Hindlimb length	54.43	0.01	45.56
Maximum distance	61.25	5.29	33.46
Sprint speed	83.61	7.30	9.09

trary, our results indicated that hatchling traits were not affected by a wide range of variance of fluctuating temperatures ( $\pm 3^\circ\text{C} \sim \pm 7^\circ\text{C}$ ) in *E. chinensis*, though the sample size is low in this study. This discrepancy may represent inter-specific differences in embryonic response to fluctuating temperatures.

It is worth mentioning that most eggs incubated at fluctuating temperatures in our study hatched successfully, even though these eggs briefly experienced extremely low and high temperatures ( $20\text{--}34^\circ\text{C}$ ) each day. Such temperatures induce high embryonic mortality when the eggs were incubated at constant temperatures (Ji & Zhang, 2001). This result supports the conclusion that exposure of eggs to adversely low or high temperatures for short periods may not increase embryonic mortality (Overall, 1994; Andrews *et al.*, 1997; Du & Ji, 2003).

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## REFERENCES

- Andrews, R. M., Mathies, T. & Warner, D. A. (2000). Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetological Monographs* **14**, 420-431.
- Andrews, R. M., Quall, C. P. & Rose, B. R. (1997). Effects of low temperature on embryonic development of *Sceloporus* lizards. *Copeia* **1997**, 827-833.
- Ashmore, G. M. & Janzen, F. J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182-188.
- Burger, J. (1990). Effects of incubation temperature on behavior of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *Journal of Herpetology* **24**, 158-163.
- Deeming, D. C. & Ferguson, M. W. J. (1991). Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: *Egg Incubation, its Effect on Embryonic Development in Birds and Reptiles*, pp.147-171. Deeming, D. C. & Ferguson, M. W. J. (eds.), Cambridge University Press, Cambridge.
- Doody, J. S. (1999). A test of the comparative influences of constant and fluctuating incubation temperatures on phenotypes of hatchling turtles. *Chelonian Conservation and Biology* **3**, 529-531.
- Du, W. G. & Ji, X. (2003). The effects of thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*. *Journal of Thermal Biology* **28**, 279-286.
- Du, W. G., Shou, L. & Liu, J. K. (2003). The effect of incubation temperature on egg survival, hatchling traits and embryonic use of energy in the blue-tailed skink, *Eumeces elegans*. *Animal Biology* **53**, 27-36.
- Janzen, F. J. & Paukstis, G. L. (1991). Environmental sex determination in reptiles: ecology, evolution, and experimental design. *The Quarterly Review of Biology* **66**, 149-179.
- Ji X. & Zhang, C. H. (2001). Effects of thermal and hydric environments on incubating eggs, hatching success, and hatchling traits in the Chinese skink (*Eumeces chinensis*). *Acta Zoologica Sinica* **47**, 256-265.
- Ji X. & Du W. G. (2001). Effects of thermal and hydric environments on incubating eggs and hatchling traits in the cobra, *Naja naja atra*. *Journal of Herpetology* **35**, 186-194.
- Overall, K. L. (1994). Lizard egg environments. In: *Lizard Ecology: Historical and Experimental Perspectives*, pp. 51-72. Vitt, L. J. & Pianka, E. R. (eds.), Princeton University Press, Princeton.
- Packard, G. C. & Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In: *Biology of the Reptilia vol.16A* pp. 523-605. Gans, C. & Huey, R. B. (eds.), Liss, New York.
- Phillips, J. A., & Packard, G. C. (1994). Influence of temperature and moisture on eggs and embryos of the white-throated savanna monitor *Varanus albigularis*: implications for conservation. *Biological Conservation* **69**, 131-136.
- Shine, R. (2004). Seasonal shifts in nest temperature can modify the phenotypes of hatchling lizards, regardless of overall mean incubation temperature. *Functional Ecology* **18**, 43-49.
- Shine, R., Elphick, M. J. & Harlow, P. S. (1997). The influence of natural incubation environment on the phenotypic traits of hatchling lizard. *Ecology* **78**, 2559-2568.

Shine, R., Madsen, T., Elphick, M. J. & Harlow, P. S. (1996). The influence of nest temperatures and maternal thermogenesis on hatchling phenotypes of water pythons. *Ecology* **78**, 1713-1721.

Van Damme, R., Bauwens, D., Braña, F. & Verheyen, R. F. (1992). Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**, 220-228.

Zhao, E. M. & Adler, K. (1993). *Herpetology of China*. Society for the Study of Amphibians and Reptiles. Ohio.

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## BOOK REVIEW

*Biology, Husbandry and Medicine of the Green Iguana*. E. R. Jacobson ed. (2003). 188 pp. Krieger Publishing Co., Florida. £35.50 (cloth).

If you remember *Iguanas: A Guide to their Biology and Captive Care* by Frye and Townsend, imagine that book greatly expanded with sections on wild iguana behaviour and iguana development, reams of unpublished information, and re-written by a suite of distinguished biologists and veterinarians, and you have something approaching the title under review. But how much more do we really need to know about green iguanas? Well, it appears from this enormously informative and highly readable volume that there is a great deal any self-respecting 'iguanophile' or reptile veterinarian should be seeking to absorb and a great deal still waiting to be discovered.

The *Biology, Husbandry, and Medicine of the Green Iguana* is an encyclopaedic synopsis of pretty much all that is known about this familiar – yet even now not entirely understood – lizard. Though the varied topics covered in its ten independently authored chapters are summaries by necessity, none is too brief and each covers its respective field comprehensively with reference to the past half-century of relevant work on the species.

The first three chapters deal predominantly with the natural history of the green iguana in the wild. Chapter one concerns *Biology and Reproduction in the Wild* and does an admirable job of presenting a wealth of varied information in a very accessible way. Only in the author's discussion of distribution and variation could I find any oversight; the lack of any reference to the very important Caribbean island populations of this species. Perhaps though, this serves to highlight the dearth of information regarding these island forms. The repeated reference to *Iguana delicatissima* as being "nearly identical" to *Iguana iguana* struck a note of dissatisfaction also, for having worked with the former species for a decade I know this not to be the case. However, these are minor gripes and ones that result largely from my personal interest in the various Caribbean iguanas.

The second chapter, *Ontogeny of Captive and Wild Iguanas: From Emergence to Mating*, takes a detailed look at growth and development from incubation through to sexual maturity and reproduction, and compares data from wild and captive studies. The authors identify through their evaluation of the available data, key areas for future research including the influence of incubation conditions upon viability of hatchlings, the independent and interacting roles of visual and chemical signalling, and the relationship between juvenile social status, adult mating strategies and eventual lifetime reproductive success.

*Nutrition in the Wild* is the topic of chapter three and provides the reader with a concise overview of what is known about the diet of iguanas in the wild. It is concise mainly owing to the paucity of reliable data on the sub-

ject and this, it is proposed, is a result of the difficulty of observing iguanas undisturbed in the wild state and the tiny fraction of time (less than 1%) that they spend feeding. The chapter is also a powerful reminder to captive managers that iguanas are strict herbivores, eating predominantly leaves, and require body temperatures similar to our own if they are to digest their food adequately.

Chapters four and five cover nutrition and management in captivity and between them contribute almost one third of the book. Despite the scientific tone of this book, therefore, these two chapters alone make this book a valuable addition to the bookshelf of any serious iguana keeper as well as to those of the student and professional herpetologist. *Nutrition in Captivity*, the fourth chapter, in particular makes interesting if slightly heavy reading in its very detailed examination of nutritional requirements and the provision of diets in captivity. An alarming variation in the composition of commercial diets is revealed and yet a slightly controversial recommendation is made; that these commercial diets should be used in preference to "nutritionally incomplete" and "inadequate" salad-type diets traditionally used in zoos and by private keepers. Whilst there is no doubt that many commercially available diets allow their users to keep and breed various iguana species successfully I feel it is short-sighted to condemn more 'natural' diets as simply inadequate. Indeed, the only successful captive breeding of *Iguana delicatissima* published to date occurred in the British Isles using a rich and varied diet of predominantly leaves and a few fruits. The reluctance to adequately explore varied 'natural' diets seems to be a recent North American prejudice (Frye and Townsend don't even mention commercial diets in 1993) and is perhaps a reflection of the veterinary emphasis in the chapter rather than a balanced consideration of international progress in iguana husbandry and diets.

Interestingly the partially European authorship of the following chapter, *Husbandry and Management*, expresses their own doubts as to the wisdom of using exclusively commercial diets. They go on to provide useful information on developing a natural-type diet for captive iguanas as well as extensive recommendations on indoor and outdoor accommodation, successful breeding, incubation of eggs, and the optimal rearing of babies.

The remaining five chapters (half of the book) focus upon health and veterinary issues, namely; *Clinical Evaluation and Diagnostic Techniques; Infectious and Non-infectious Disease; Drug Dosages and Chemotherapeutics; Anaesthesia and Surgery; and Diagnostic Imaging*.

Whilst all are of great use to veterinarians, the first two chapters are also of particular interest to zoo and private iguana keepers. These chapters provide, respectively, detailed technical but readily understandable explanations of the processes of health assessment and diagnosis; and of the more common ailments of the ani-

mal in captivity including viral, bacterial and fungal infections, internal and external parasites, nutritional and reproductive disorders, and environmental and stress related injuries and illnesses. The latter three chapters are unlikely to be extensively thumbed by anyone other than veterinarians but may come in very useful to the private iguana keeper whose local vet lacks either experience or confidence with such a comparatively exotic patient.

Each chapter concludes with its own, invariably extensive, reference list and the whole book benefits from a detailed index. There are sixteen pages of colour plates, the vast majority of which show injuries and illnesses or the treatments and surgery necessary to correct them. The exception to these veterinary images is a handful of 'typical iguana' shots demonstrating sexual dimorphism, including femoral pore size, and a sequence of what appears to be some really very poor iguana accommodation. This is the only really disappointing aspect of the book. Though half the text is dedicated to describing the wonderful and diverse habi-

tats, complex behaviours, varied diet, and demanding husbandry of this photogenic lizard there is no visual record of any of this wonderful research or diagrammatic or photographic guidance to setting up the complex accommodation described in Chapter 5. Such high quality and up to date text surely deserved photographs of relevant subject matter and equally high quality.

*Biology, Husbandry, and Medicine of the Green Iguana* is an impressive compilation of information about every aspect of green iguanas by an equally impressive line up of researchers. Unless you are a devoted iguana keeper or veterinarian however, you are unlikely to need such a detailed monograph on your bookshelf, but for those that fall within these categories, this is an invaluable reference.

*Richard Gibson*  
*Zoological Society of London*

# THE HERPETOLOGICAL JOURNAL

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(revised January 2004)

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Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* 2, 206–210.

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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