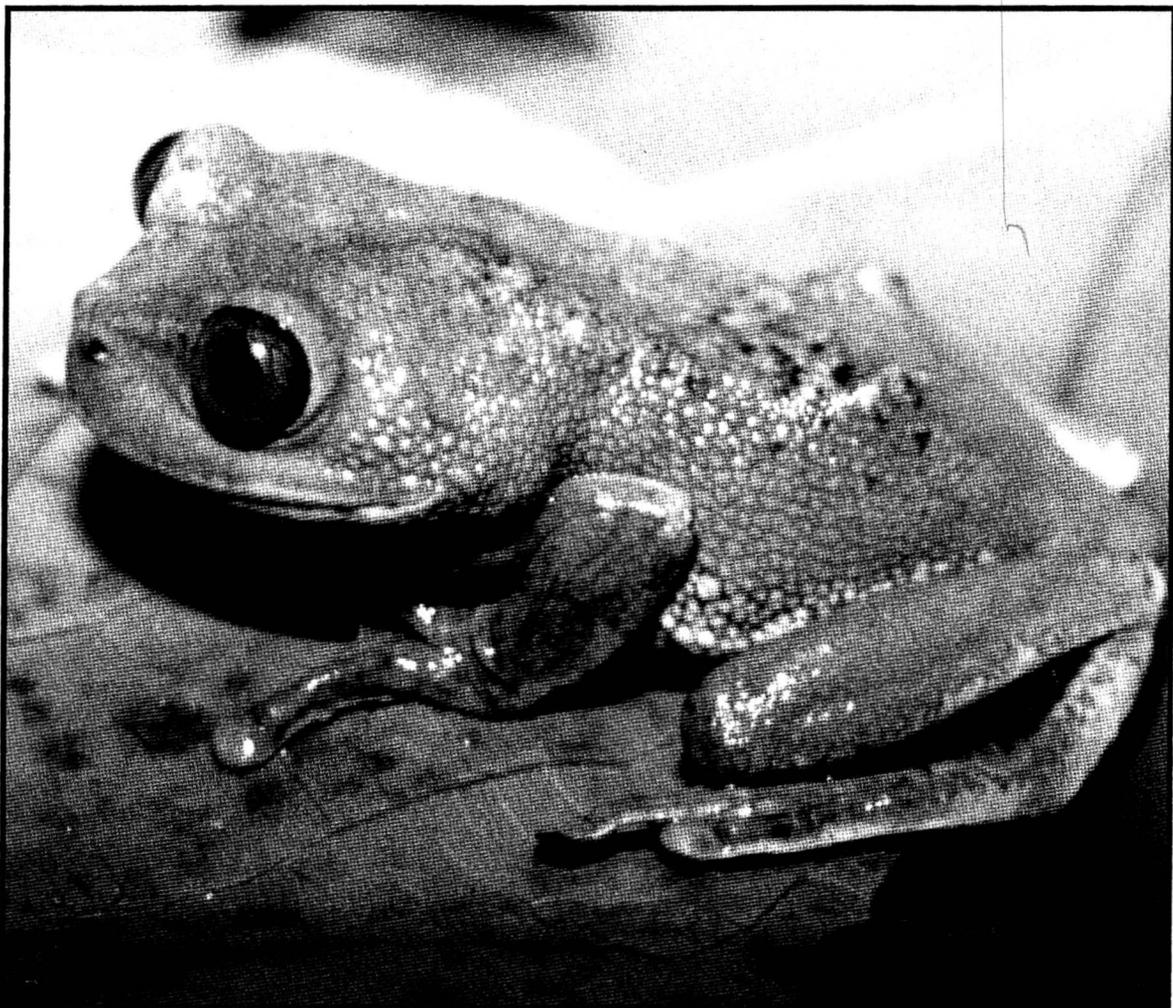


Volume 15, Number 3

July 2005
ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

The Herpetological Journal is published quarterly by the British Herpetological Society and is issued free to members. Articles are listed in *Current Awareness in Biological Sciences*, *Current Contents*, *Science Citation Index* and *Zoological Record*.

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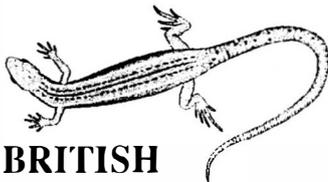
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FRONT COVER: *Leptopelis crystallinorum* (V. Gossmann)

SIZE AT MALE MATURITY, SEXING METHODS AND ADULT SEX RATIO IN LOGGERHEAD TURTLES (*CARETTA CARETTA*) FROM ITALIAN WATERS INVESTIGATED THROUGH TAIL MEASUREMENTS

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Tail length is the main secondary sexual characteristic of adult sea turtles. In order to assess the size at which sexual dimorphism in this character becomes evident, six different measurements of tail length were collected or calculated from 2631 *Caretta caretta* specimens found in the waters around Italy. These data show that an average male develops a longer tail at a size of 70 cm and attains sexual maturity at a size of 75-80 cm Curved Carapace Length. Studies of adult sex ratio based on tail measurements should therefore be restricted to specimens >75 cm. The distance from the cloaca to the posterior margin of the carapace appears to be the most effective measurement for sexing turtles of this size among the six characteristics investigated. In the sample, females are estimated to comprise 61% of the specimens >75 cm.

Key words: male sexual maturity, Mediterranean Sea, sexing method, sexual dimorphism

INTRODUCTION

The loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758), is globally classified as an endangered species (Hilton-Taylor, 2000). Population sub-structuring and genetic isolation – at least at the female level (Schroth *et al.* 1996, Laurent *et al.* 1998) – make Mediterranean loggerheads vulnerable to numerous anthropogenic threats, particularly high fishing effort. Therefore, understanding the population dynamics and demography of these animals are important for any effective regional conservation strategy. In this context, sex ratio and age/size at maturity are among the most important demographic parameters. In the Mediterranean, a few investigations on sex ratio at sea have been carried out on juveniles (Casale *et al.*, 1998; Casale & Freggi, in press; Lazar *et al.*, in press) but no data are available for adults. Size at maturity of females can be inferred from data on nesting females (Margaritoulis *et al.*, 2003), but size at maturity of Mediterranean males is unknown.

Different methods are available to obtain sex ratios in hatchling, juvenile and adult sea turtles (review in Wibbels, 2003). Although it requires the sampling of turtles at sea, adult sex ratio is commonly regarded as relatively easy to obtain because adults are sexually dimorphic. In particular, the most obvious characteristic is the large and muscular prehensile tail of adult males (Wibbels, 1999). However, using tail length for diagnosing the sex makes an important, but often implicit assumption: i.e. a turtle with a 'short' tail is more likely to be a female than an immature male. This assumption can be satisfied if the sample includes only turtles above

the average size of adult males or the average size in which males begin to show an evidently longer tail. Unfortunately, average or even minimum size of nesting females are usually used as threshold size of specimens in such studies, because size at maturity of males is much more difficult to know. Hence, this lack of knowledge bears the potential risk of underestimating the number of males, particularly if they mature at a size larger than the average or minimum size of nesting females.

A second problem of using tail length for diagnosing sex is to have a meaningful measurement and a rigorous threshold value. On the contrary, usually thresholds are arbitrary and both they and measurements vary depending on the researcher. Furthermore, these methods are apparently not based on any specific study. The most widespread measures – used with constant arbitrary thresholds differing between researchers – are 'Plastron Tip of Tail' and 'Carapace-Tip of Tail'; furthermore, according to Bolten (1999) adult males would have a higher 'Plastron-Tip of Tail' to 'Cloaca-Tip of Tail' ratio than adult females.

The aim of the present study, based on a large sample, is to (1) detect the size at which sexual dimorphism in tail length becomes evident, and thus the size at male sexual maturity; (2) investigate which type of measure and threshold value are the most appropriate ones for sexing adult/maturing turtles; and (3) provide an adult sex ratio for the study area.

MATERIALS AND METHODS

As part of a research and conservation programme (Argano, 1992) we collected biometric data from 2631 live *Caretta caretta* specimens ranging from 17 to 109

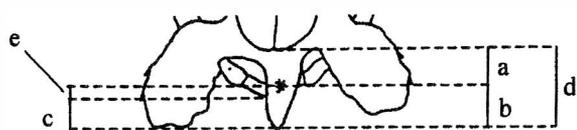


FIG. 1. Tail measurements. Measured: a, Plastron-Cloaca; b, Cloaca-Tail; c, Carapace Tail. Calculated: d, Plastron-Tail; e, Carapace-Cloaca. See materials and methods for details.

cm CCLn-t from 1981-2003. These turtles were found in different circumstances (incidentally caught by longline, trawl, nets, and other fishing methods; taken from the sea surface by hands or handled tools; stranded; found while nesting; seized; unknown origin). These data included curved carapace length notch to tip (CCLnt; Bolten, 1999) and three measurements of tail: (1) distance from the posterior margin of the plastron to cloaca (Plastron-Cloaca; Fig. 1a), (2) distance from cloaca to the tip of the tail (Cloaca-Tail; Fig. 1b), (3) distance from the posterior margin of the carapace to the tip of the tail (Carapace Tail; Fig. 1c). From these three tail measurements we calculated three other values: (4) distance from the posterior margin of the plastron to the tip of the tail (Plastron-Tail; Fig. 1d), (5) distance from the

posterior margin of the carapace to the cloaca (Carapace-Cloaca; Fig. 1e), and (6) Plastron-Tail to Cloaca-Tail ratio (Plastron-Tail/Cloaca-Tail). In relationships between carapace length and tail parameters, for each 5-cm carapace length class we calculated the upper value including 99% of the sample as mean + 2.33 SD; we used only classes with a reasonable number of specimens (min = 48).

Ten turtles were certainly adult females, because they were found while nesting or they were recaptured at sea with tags identifying them as females which nested previously (D. Margaritoulis, pers. comm.). These specimens were excluded from the sex ratio estimation, because their sampling was not independent of their sex. 95% Confidence Intervals of sex ratios were calculated according to the method for binomial distributions (Zar, 1999).

RESULTS AND DISCUSSION

All parameters except 'Plastron-Tail/Cloaca-Tail' showed a strong difference between specimens above and below 65 cm CCLnt (Fig. 2). From the 65-70 size class onward, more specimens with high values were

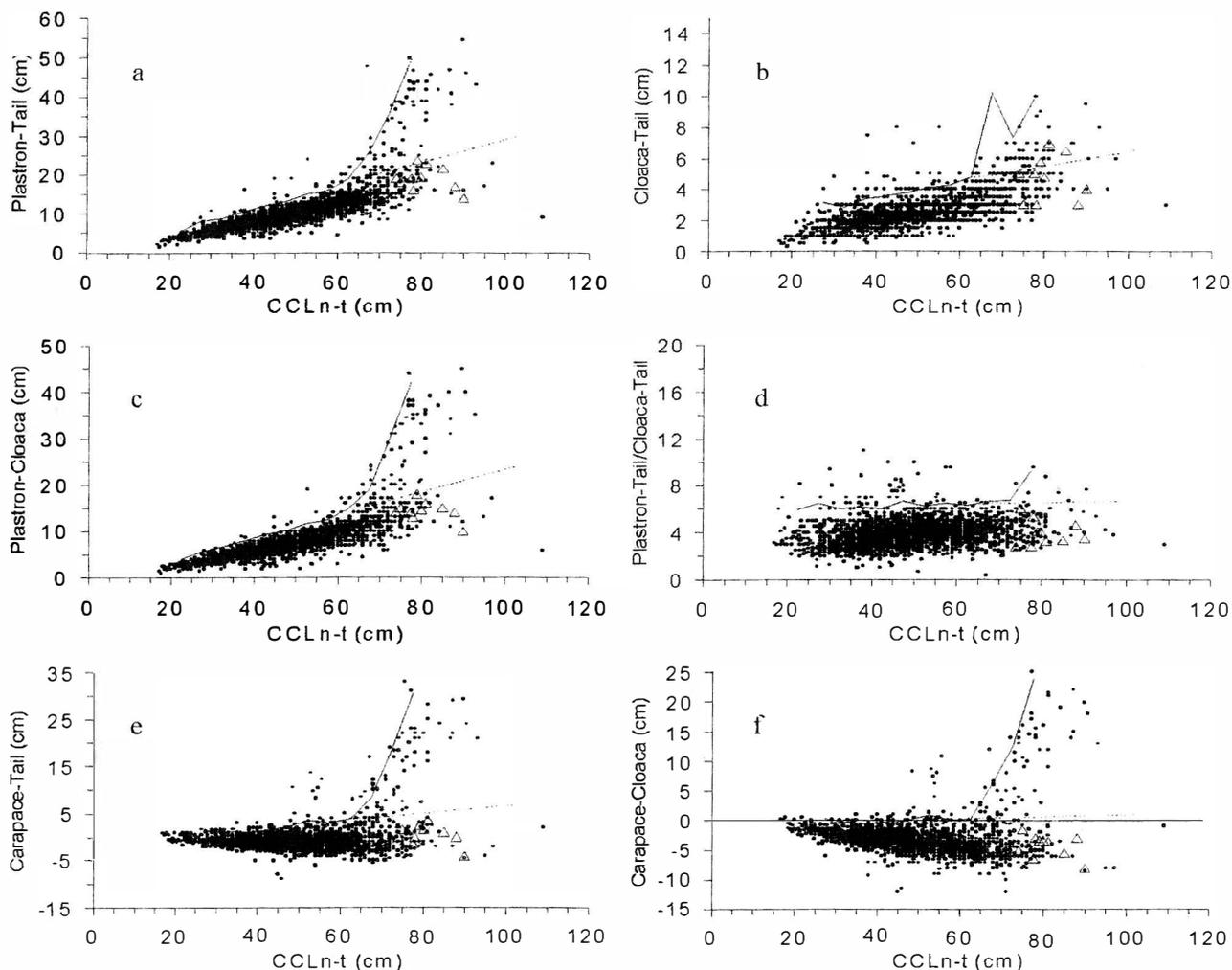


FIG. 2. Relationships between tail-related measures and carapace length ($n=2631$). Solid line includes 99% of the sample (see text). Dotted line is its tendency in the range >65 cm based on values in the range <65 cm. Triangles represent adult females.

observed than expected from the distribution in the previous size classes. This suggests that an average Mediterranean male begins to develop an elongated tail around a size of 70 cm CCLn-t and that full maturity is probably attained around 75-80 cm, the first size class in which tail lengths reach maximum values. Therefore, in the Mediterranean, only loggerheads with CCLn-t >75 cm should be used for sex ratio estimates based on tail measurements.

Limpus (1990) found that in Heron Island Reef (Queensland) the average loggerhead female starts breeding at a size slightly smaller than the average size of nesting females. The means observed in Greece, hosting the main known nesting sites in the Mediterranean, are around 83 cm CCLn t (Margaritoulis *et al.*, 2003). If the rule above applies to the Mediterranean populations too, present findings do not suggest a strong difference between male and female size at maturity.

The six tail parameters investigated show different characteristics affecting their efficacy for sexing turtles. 'Plastron-Tail' (the whole 'tail'; Fig. 2a) can be separated into two measures: 'Plastron Cloaca' and 'Cloaca Tail'. On the basis of specimens known to be adult females and the lack of an evident clustering, the increasing of 'Cloaca-Tail' observed in large-size specimens appears to be independent of sex (Fig. 2b), in contrast to 'Plastron-Cloaca' (Fig. 2c). Thus, 'Plastron-Cloaca' seems to be a better indicator of sex than 'Cloaca Tail' and 'Plastron Tail'. Generally, measures considering the cloaca (like 'Carapace-Cloaca') are expected to be better indicators of maleness than those considering the tip of the tail.

Probably for the same reason, the ratio based on the tip of the tail ('Plastron-Tail' to 'Cloaca-Tail'; Fig. 2d) does not show any clustering in the large size class and the specimens known to be adult females distribute across most of the range; so this ratio is unlikely to be useful for diagnosing sex.

'Plastron Cloaca' (Fig. 2c) (and 'Plastron Tail'; Fig. 2a) is positively correlated to carapace length in all size classes; in presumed males (higher cluster) this relationship would just have a different degree. Hence, a sexing threshold for this measurement should be a size dependent variable, and not a constant, otherwise large females would be wrongly diagnosed as males. On the contrary, it seems that 'Carapace Tail' (Fig. 2e) remains constant and 'Carapace Cloaca' (Fig. 2f) even decreases as size increases, except for presumed males, where it would change in a positive relationship. So, using only one threshold for the whole large size class would be appropriate with these two measures, which are so preferable to 'Plastron Cloaca'. Good candidates as sexing thresholds are the values 5 and 0 cm for 'Carapace Tail' and 'Carapace Cloaca' respectively (Figs. 2e, 2f), which include 99% of the immature size sample as well as all known adult females of the sample. Moreover, 'Carapace Cloaca' seems to be preferable to 'Carapace Tail' because of the considerations made above concerning tail vs. cloaca, the negative correlation with size in

immatures and adult females in contrast to the positive correlation in presumed adult males, and the very convenient threshold. This threshold means that only males would have a cloaca protruding beyond the carapace, and for the field work this implies that no measurements at all need to be taken, making data collection very easy. Hence, 'Carapace Cloaca' appears to be the best of the six tail parameters taken into consideration. It is interesting that its threshold value (0 cm; Fig. 1e, 2f) is probably not arbitrary, but may have a biological reason, because possible injuries of the distal part from the cloaca (the true tail) are less harmful than ones on the proximal part, and females would have no advantage balancing the disadvantage of exposing a vulnerable part beyond the protection of the carapace. On the other hand, males have the fundamental need to be able to mate.

Although measuring tail length cannot be so accurate as laparoscopy in diagnosing sex of large specimens (i.e. those thought to be adults), nonetheless it is easy to apply in any field condition and can provide a large amount of data, provided that a good method is used. In summary, present results suggest 'Carapace Cloaca' as the best of the six tail parameters taken into consideration, and in the Mediterranean it should be used on specimens larger than 75 cm only.

With the method above a female proportion of 61% is calculated for specimens larger than 75 cm found at sea in the study area ($n=69$). However, adult sex ratio can be biased by sex specific patterns of reproductive migration, and to avoid this bias it is preferable to estimate sex ratios during nonmigratory periods only (Wibbels, 2003). So we calculated sex ratios for two periods: a 'warm' one (Apr-Sep), when reproductive migration occurs, and a 'cold' one (Oct-Mar), when reproductive migrations are unlikely to occur. The resulting sex ratios were 53.8% (95% CI: 39.5-67.8%; $n=52$) for the warm period and 76.5% (95% CI: 50.1-93.2%; $n=17$) for the cold one. Although the two sex ratios are not significantly different (Fisher exact test: $P=0.15$; $n=69$) possibly due to the small sample size, we conservatively considered the cold period sex ratio more representative of the real one, which would be likely to be skewed towards females.

This finding is apparently in contrast with unbiased sex ratios recently reported by two studies on loggerhead turtle juveniles in the Mediterranean (Casale & Freggi, in press; Lazar *et al.*, in press), but this difference could be explained by male-biased juveniles coming from the Atlantic (Casale *et al.*, 2002).

ACKNOWLEDGEMENTS

The data here presented were collected thanks to the many individuals who participated in the "Progetto Tartarughe" (Univ. Roma/WWF Italy), and especially: G. Gerosa (Chelon), M. Cocco (WWF-Italy), A. Dominici and S. Nannarelli (Hydrosphera), A. Donato (Università di Messina). Special thanks to the many fishermen who collaborated with the project.

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Accepted: 27.7.04

A NEW LARGE TREE FROG FROM NORTH-WESTERN GABON (HYPEROLIIDAE: *LETOPELIS*)

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A new large, green species of *Leptopelis* from the Monts de Cristal, north-western Gabon, is described. It is similar to the sympatric *L. brevirostris*. The most striking character to distinguish these two taxa is the absence of a tympanum in the new species in contrast to the presence of a tympanum in *L. brevirostris*.

Key words: Africa, Anura, *Leptopelis brevirostris*, systematics

INTRODUCTION

Little is known about the amphibians from most parts of western Central Africa (Poynton, 1999). Field surveys by different workers at the Monts de Cristal, north-western Gabon, revealed several new country records and species new to science. Currently, we are aware of about 50 species of anurans from this region (Lötters *et al.*, 2001; Rödel & Pauwels, 2003; unpubl. data). The genus *Leptopelis* Günther, 1859 contains 49 recognized species, spread out through most of sub-Saharan Africa as far south as the eastern Cape Province in South Africa (Frost, 2002). Our amphibian collections from the Monts de Cristal comprise six *Leptopelis* forms (see below). One *Leptopelis* specimen, a large, green arboreal frog, is not assignable to any described species (e.g. Perret, 1958, 1962, 1973; Laurent, 1973; Largen, 1977; Amiet, 1991, 2001). This specimen is most similar to *L. brevirostris* (Werner, 1898), a species also represented in our collection from the Monts de Cristal. However, it differs in several characters from *L. brevirostris*, especially the absence (versus presence) of a tympanum. We thus regard it as a representative of a large *Leptopelis* species new to science.

MATERIAL AND METHODS

We examined material from MHNG (Muséum d'Histoire Naturelle, Genève), IRSNB (Institut Royal des Sciences naturelles de Belgique, Brussels: field numbers of O.S.G. Pauwels), RMCA (Royal Museum for Central Africa, Tervuren), ZFMK (Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn), ZMB (Zoologisches Museum der Humboldt-Universität, Berlin) and MOR (research collection of M.-O. Rödel: field numbers of O.S.G. Pauwels). Specimens investigated for comparison are listed in the Appendix.

The holotype of the new species was preserved in 70% ethanol after taking colour slides for the life colour description. To determine sex and reproductive status as

well as to examine if a tympanum is hidden behind thickened skin, the holotype was dissected. We took measurements with dial callipers (± 0.1 mm). The snout-vent length is abbreviated SVL. Drawings were done with the aid of a camera lucida, attached to a dissecting microscope (Zeiss Stemi SV 6). Webbing formulae follow the system used by Glaw & Vences (1994).

A finger tip was removed as a tissue sample and used to sequence a 500 bp fragment of mitochondrial DNA, using the gene 16S rRNA gene. For methods used see Lötters *et al.* (2004).

DESCRIPTION

LETOPELIS CRYSTALLINORON SP. N. (FIGS. 1; 2A,B,E,G,H)

LETOPELIS BREVIROSTRIS (NON WERNER, 1898):
LÖTTERS *ET AL.*, 2001: 32.

Holotype. ZFMK 73139 (field number SL 1061), adult female, in forest in the vicinity of the Barrage de Tchimbélé (ca. 0°37'N, 10°24'E; about 560 m above sea level), Monts de Cristal, Gabon, 22 January 2000, M. Frankenstein, V. Gossmann, S. Lötters and F. Obame.

Diagnosis. An arboreal *Leptopelis* (sensu Schiøtz, 1999) resembling *L. brevirostris*, with (1) adult female SVL about 57 mm; (2) head wider than long; (3) eye relatively large with horizontal eye diameter almost twice the distance from nostril to anterior corner of eye; (4) dorsal snout shape truncate; (5) tympanum absent; (6) dorsal skin granular; (7) foot webbing formula as far as known 1(0), 2i+e(½), 3i(1) 3e(½), 4i(1) 4e(½), 5(0), hand webbing formula 1(1½), 2i(1) 2e(½), 3i+e(1), 4(1); (8) well developed subarticular tubercles and terminal discs present on all toes and fingers; (9) colour in life dorsally brilliantly green with a few diffused tan to brown markings; no white spot present below eye (often present in several other *Leptopelis* species); laterally whitish-tan spots; ventrally creamy white, iris bronze with a black ring at periphery; (10) vomerine teeth massive and fused to U-shaped structure; (11) sequence a 500 bp fragment of the mitochondrial 16S rRNA gene as stored at GenBank (accession number AY702652).

DESCRIPTION OF HOLOTYPE

Adult female in non-reproductive state; body robust (Fig. 1), head narrower than body and head width; snout appears truncate in dorsal view (Fig. 2b), and rounded in lateral view (Fig. 2a); maxillary and vomerine teeth present, the latter massive and fused to U-shaped structure (Fig. 2e); choanae rounded; tongue as long as wide, free for about half its length; nares lateral, visible from dorsal view (Fig. 2a,b); canthus rostralis straight; loreal region slightly concave; eye relatively large with horizontal eye diameter almost twice the distance from

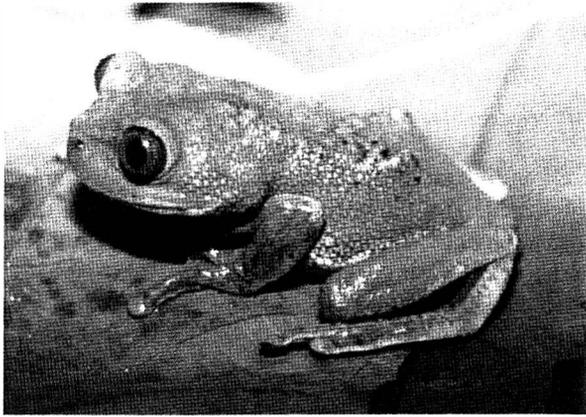


FIG. 1. Female holotype of *Leptopelis crystallinoron* in life (ZFMK 73139). Note absence of tympanum. Photo: V. Gossmann.

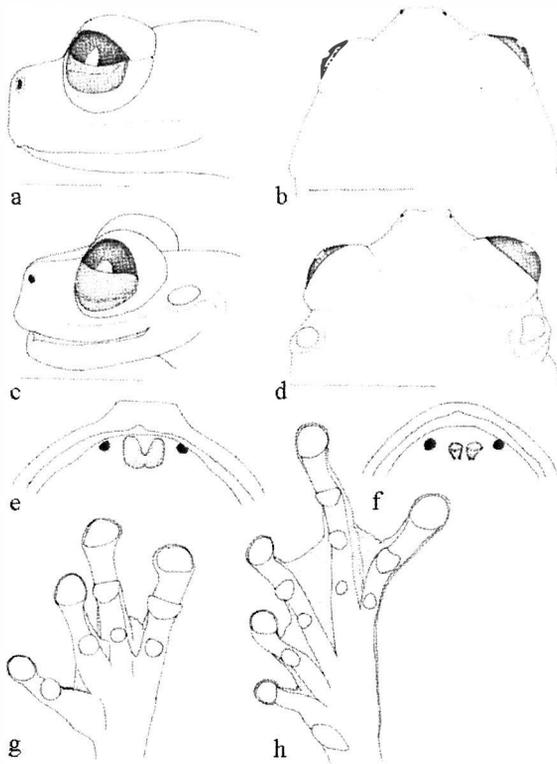


FIG. 2. Morphological aspects of *Leptopelis crystallinoron* (female holotype, ZFMK 73139) and *L. brevirostris* (female, MOR-P578): lateral and dorsal views of head of *L. crystallinoron* (a, b) and *L. brevirostris* (c, d); position and shape of vomerine teeth in *L. crystallinoron* (e) and *L. brevirostris* (f), and ventral views of left hand (g) and foot (h) of *L. crystallinoron*. Scale bar = 1 cm.

nostril to anterior corner of eye; pupil vertical; tympanum absent (Fig. 2a,b), not even hidden; absence from behind thickened skin confirmed by dissection; skin of all surfaces granular (except parts of inner leg and arm), most prominently so at the angles of jaws, and on head and flanks; hind limbs relatively long with tibia length reaching almost half SVL; foot webbing formula 1(0), 2i+e(½), 3i(1) 3e(½), 4i (1) 4e (½), 5(0), all toes with lateral fringes; relative length of toes: I < II < V = III < IV; outer metatarsal tubercle absent, inner well developed, ovoid; rest of sole tubercular; well developed subarticular tubercles at joints of most phalanges of toes, with most distal subarticular tubercle on toe IV and V each slightly bifid; tips of all toes with discs, each about 1.5 times wider than width of adjacent phalange; hand webbing formula 1(1½), 2i(1) 2e(½), 3i+e(1), 4 (1), all fingers with lateral fringes; relative length of fingers: I < IV < II < III, Finger I < Finger II when adpressed; outer metacarpal tubercle absent, inner weak, rounded; rest of sole tubercular; well developed subarticular tubercles at joints of most phalanges of fingers, with most distal subarticular tubercle on Finger III and IV each slightly bifid; tips of all fingers with discs, each about 1.5 times wider than width of adjacent phalange.

In life, the holotype (see Lötters *et al.*, 2001: 31) was overall brilliantly green dorsally and creamy white ventrally. Few diffused tan to dark brown markings were present on the back, outer extremities and around the cloacal region. Outer parts of feet and arm were whitish. The lower parts of the flanks had white spots. Discs on toes and fingers had a more clear green than the dorsum. The area around the nares was dark brown. Lips were pale. The iris was bronze with a black ring around it. In preservative, all dorsal surfaces became brownish tan and all ventral surfaces brownish.

Measurements (mm) and ratios. SVL, 56.9; head length from tip of snout to angle of jaws, 20.3; head width at angles of jaws, 25.3; interorbital distance, 7.4; distance between nares, 4.3; distance from nostril to anterior corner of eye, 4.3; horizontal eye diameter, 7.2; tibia length, 25.2; foot length from tip of longest toe to proximal outer metatarsal tubercle, 24.1; hand length from tip of longest finger to proximal outer metacarpal tubercle, 17.2; head length/SVL, 0.4; head length/head width, 0.8; head width/SVL, 0.4; distance from nostril to eye/eye diameter, 0.6; tibia length/SVL, 0.4.

Etymology. The specific name is the Latinized form of the Greek words *krystallinos*, (=crystal) and *oroon* (=mountains), thus referring to the region in which the new species was found, the Monts de Cristal.

DISTRIBUTION AND ECOLOGY

Leptopelis crystallinoron is known only from the type locality in the central Monts de Cristal and thus falls within the recently declared Monts de Cristal National Park. The only specimen available was collected in altered but good standing forest, while active at 1–3 m

above ground during night time. There was apparently no open water in close vicinity. Other species of *Leptopelis* known from the same general area include *L. aubryi* (Duméril, 1856), *L. brevirostris*, *L. calcaratus* (Boulenger, 1906), *L. millsoni* (Boulenger, 1894) and *L. rufus* (Reichenow, 1874).

The female holotype contained numerous yellow ovarian eggs, not ready for oviposition. The stomach was empty. The rectum was completely filled with a brown unidentifiable mass and also contained several (>10) nematodes.

DISCUSSION

The characters that distinguish between members of the genus *Leptopelis* are life style, adult SVL, skin texture, webbing, subarticular tubercles, colour and pattern (e.g. Schiøtz, 1999). We add another character, the absence versus presence of a tympanum. So far, *L. crystallinoron* is the only member of the genus in which a tympanum is absent. In some East African species (e.g. *L. barbouri* Ahl, 1929; *L. karissimbensis* Ahl, 1929; *L. parkeri* Barbour & Loveridge, 1928; *L. uluguruensis* Barbour & Loveridge, 1928) the tympanum may be very small and indistinct but not absent (Schiøtz, 1999).

The new species can readily be distinguished from all other known *Leptopelis* species by a combination of characters which include head wider than long, dorsal snout shape truncate, tympanum absent, granular skin, foot well webbed, toe and finger discs present and green dorsal colour in life (cf. Schiøtz, 1999). Exceptions are *L. brevirostris* from western Central Africa (see Perret 1958) and *L. brevipes* Boulenger, 1906 from Bioko probably a synonym of *L. brevirostris* (Mertens, 1965, but see Frost, 2002). The most striking difference between these two forms and *L. crystallinoron* is the presence (versus absence in *L. crystallinoron*) of a clearly distinct, obliquely placed tympanum (Fig. 2a-d). We examined 45 specimens of *L. brevirostris* from different localities (see Appendix 1) scattered throughout its geographical range (cf. Frost, 2002) including the holotype and material from the Monts de Cristal in Gabon (i.e. the region from which we here describe *L. crystallinoron*). In all of them, the tympanum was well developed. In addition, *L. brevirostris* appeared to be more slender with a less granular dorsum. Another striking difference was the completely different shape of vomerine teeth (Fig. 2e,f). However, more material needs to be examined (especially of *L. crystallinoron*) to confirm the latter differences.

A comparison of sequences each of a 500 bp fragment of mitochondrial DNA of the 16S rRNA gene of the new species and *L. brevirostris* (ZFMK 72065, GenBank accession number AF215447), revealed that they are closely related. The samples compared differed in 14 bp, which equals a substitution level of 2.8 %.

Leptopelis brevirostris is believed to be a snail-feeding specialist (Perret, 1966), and so might be *L. crystallinoron* as is suggested by the presence of massive fused vomerine teeth. However, the stomach of the

holotype was empty and several anatomical adaptations to snail eating (cf. Drewes & Roth, 1981) were absent.

Leptopelis crystallinoron is likely to be endemic to the Monts de Cristal, firstly because it has not been recorded from other regions despite several surveys, and secondly because the Monts de Cristal may represent a regional centre of endemism (e.g. Cribb *et al.*, 1989; Sosef, 1994; Rödel & Pauwels, 2003; own unpubl. data). In contrast, *L. brevirostris* is widely distributed in southern Nigeria, Cameroon, Equatorial Guinea, Gabon, northern Republic of Congo and western Central African Republic (cf. Frétey & Blanc, 2000; Frost, 2002).

ACKNOWLEDGEMENTS

The Centre national de Recherche et Technologie (CENAREST) of the Libreville University issued collection and export permissions. We are especially grateful to H. Bouroubou and P. Posso from the Libreville University for this support. Most valuable logistic support was provided by A. Kamdem Toham of the WWF Ecoregion Program and J. N. Cavallero of Société d'Énergie et d'Eaux du Gabon (SEEG). Field trips were shared with M. Frankenstein, V. Gossmann and F. Obame. The 'Alexander Koenig Stiftung' at ZFMK kindly provided financial support to the field work by the senior author and colleagues. Support from the African Biodiversity Information Centre (ABIC) at RMCA (Royal Museum for Central Africa, Tervuren), through a framework agreement of the latter institution with the Belgian Development Cooperation, enabled M. Burger to examine comparative material at RMCA. O. S. G. Pauwels (IRSNB) kindly made available material collected by him in Gabon. We thank W. Böhme (ZFMK), R. Günther (ZMB), D. Meirte (RMCA) and A. Schmitz (MHNG) for providing working possibilities in their respective collections. J. Kosuch (Mainz University) kindly ran the genetic analysis of the new species.

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Accepted : 1.10.04

APPENDIX I

SPECIMENS EXAMINED FOR COMPARISONS *Leptopelis brevirostris* (45 specimens)

GABON: MHNG 2214.14-15, Makokou; IRSNP P757, Tchimbélé, Monts de Christal; IRSNB P662, Moudouma, Massif du Chaillu; MOR P578, Itsiba, Massif du Chaillu.

CAMEROON: MHNG 1545.77, 1545.92, Yaoundé; ZFMK 61305-311, 61313, Mt. Cameroon; MHNG 1545.86, Mt. Manengouba; MHNG 1545.80-85, Nkondjock; MHNG 1545.90-91, Ototomo; MHNG 1545.89, Njazeng; MHNG 2094.23, Nlong; MHNG 1545.78-79, 1545.87, Yungué; MHNG 1545.88, ZFMK 1906, Kribi; MHNG 953.25-26, 993.54-60, Foullassi; RMCA 77-32B-4, SE Cameroon; RMCA 73-18-B-55, Eboga River, Nyona; ZMB 13913 (holotype), Victoria; ZFMK 72065-066, no exact data.

CAN AGGREGATION BEHAVIOUR OF *PHRYNOMANTIS MICROPS* TADPOLES REDUCE PREDATION RISK?

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In the Comoé National Park in Ivory Coast, West Africa, tadpoles of the microhylid frog *Phrynomantis microps* often stay in large and densely packed aggregations near the water surface of savanna ponds. Previous studies have shown that aggregation behaviour was initiated by the presence of visually guided aquatic predators. In the present study, I investigated how efficiently aggregation of *P. microps* tadpoles reduces the risk of predation. I used an experimental design to count the number of attacks by predators on *P. microps* tadpole at different densities and distributions. The total strike rate of predators was significantly lower when *P. microps* tadpoles were aggregated than when they were randomly distributed. However, per capita strikes rate did not differ between treatments. Further replicate trials might have detected benefits to the individual tadpole from aggregating.

Key words: anti-predation behaviour, anurans, dilution effect, social behaviour

INTRODUCTION

Animals of many species live together in groups for the whole life span or, more often, for a particular period. Group members may experience increased foraging efficiency (Beiswenger, 1975), prevention of desiccation (Heinen, 1993), increased reproduction possibilities (Ryan *et al.*, 1981) or reduced risk of predation (Hamilton, 1971; Bertram, 1978; Pulliam and Caraco, 1984). There are three different ways in which grouping may help to reduce rates of successful attack by predators. The dilution effect is a numerical phenomenon with the result that in a larger group an individual has a lower risk of being taken (Foster & Treherne, 1981; Turner & Pitcher, 1986; Coster-Longman *et al.* 2002). The confusion effect provides safety by increasing the number of capture mistakes by predators against a larger group size of the prey (Milinski, 1979; Landeau & Terborgh, 1986; Krakauer, 1995). The Trafalgar effect reduces risk of predation in groups by cooperative behaviours of the prey such as abrupt changes in speed of movement or rapid disappearance of the aggregation after only a few members of the group detect a predator (Treherne & Foster, 1981).

Numerous studies have shown that one or more of these three effects are responsible for the aggregation behaviour in any particular species, but only a few experimental studies have quantified the individual survival rate of prey corresponding to their distribution pattern (e.g. Watt *et al.*, 1997).

In the Comoé National Park, West Africa, *Phrynomantis microps* tadpoles form aggregations near the surface of ephemeral savanna ponds (Rödel & Linsenmair, 1997; Spieler, 2003). Previous experiments in containers with clear water demonstrated that the aggregation behaviour of *P. microps* tadpoles was only shown while predators were present and was induced by

visual detection of the predator or by chemical cues (Rödel & Linsenmair, 1997). Each tadpole in such an aggregation floated nearly motionless until disturbed by a predator attack. Then, all tadpoles quickly dispersed, triggered by initial swimming activity from a few tadpoles close to the centre of the disturbance.

In this study, I used an experimental design to investigate how efficiently the aggregation of *P. microps* tadpoles reduces the risk of being preyed upon. The design was chosen to test only the dilution hypothesis.

METHODS

STUDY AREA

The study area is situated in the Guinean savanna of Comoé National Park (8°5'-9°6' N, 3°1'-4°4' W) in the north-eastern part of the Ivory Coast (West Africa). This region is characterized by distinct wet and dry seasons, with a mean annual precipitation between 750 and 1100 mm during 1994 and 2001 (measurements of the research camp in Comoé National Park). The core dry period, lasting from December to February, usually lacks precipitation. Most bodies of water in the study area are ephemeral ponds that always dry up in the dry season. *Phrynomantis microps* breeding ponds do not usually fill before April or May.

STUDY ANIMAL

Phrynomantis microps Peters, 1875 is a medium-sized microhylid frog of the West African savanna. Breeding occurs throughout the rainy season in ephemeral savanna ponds (Rödel, 2000). The tadpoles are barely pigmented and thus very translucent (Rödel & Spieler, 2000). They are suspension feeders with a specialized filter apparatus.

GENERAL METHODS

I conducted the experiments in the research camp in Comoé National Park between 1-15 August 1999. I used a transparent plexiglass floating arena (35 × 20 × 4

cm) with 40 compartments to quantify the strike rate of predators presented with different tadpole densities and distributions. Each compartment ($4.9 \times 3.3 \times 4.0$ cm) was large enough to allow 10 medium sized *P. microps* tadpoles to maintain tadpole-tadpole distances similar to that measured in large aggregations of more than 100 individuals from natural ponds. Standard capped plastic vials used for 35 mm film were attached to two sides for buoyancy. This arrangement was similar to that used by Watt *et al.* (1997). The arena was floated in green plastic containers ($45 \times 35 \times 25$ cm) that were filled to a depth of 8 cm with clear water from rock pools and savanna ponds.

I used carnivorous tadpoles of *Hoplobatrachus occipitalis* and killifish *Nothobranchius kiyawensis* of similar size as predators. Both predator species are natural predators of *P. microps* tadpoles and show a similar hunting strategy. This made it possible to use both predators together in the experiments in view of the very low abundance of both predator species during the study period. The predators were kept in a large aquarium 24 hr before the experiment started and were fed with mosquito larvae. The feeding did not occur *ad lib* so that the predators reacted with the same intensity to the addition of new mosquito larvae. One carnivorous tadpole and one killifish were chosen randomly and transferred to each of six containers 10 hr before I presented the arena to the predators. I never saw any kind of interaction between the predatory tadpole and fish.

The experimental herbivorous *P. microps* tadpoles I used had a snout-vent length of 6.0-9.4 mm and were at developmental stages 25-36 (Gosner, 1960). These tadpoles were netted in one savanna pond and kept together in one aquarium 24 hr before the experiment started. They were fed with a suspension of algae.

Each of the 13 experiments involved testing six trials in succession during a single day. The floating arena was placed into one of the six containers and the *P. microps* tadpoles were pipetted into the arena according to one of the following treatments: (1) one tadpole, randomly placed in the arena; (2) five tadpoles, each in one compartment; (3) five tadpoles, together in one compartment; (4) 10 tadpoles, each in one compartment; (5) 10 tadpoles, together in one compartment; (6) no tadpoles. The compartments used in each arrangement were chosen randomly. Transferring 10 tadpoles took 2-3 min. After recording, the arena was cleaned, placed into the next container and another set of tadpoles were transferred using the arrangement described above. The sequence of allocation was assigned randomly.

After an acclimation period of 10 min with the predators, I recorded in each trial the number of strikes the predators made against the experimental tadpoles, making observations from a 45° angle above the arena over a 10 min period. This observer position allowed me to see through the arena with the experimental tadpoles to the base of the container where the predators were contained. Without the presence of prey, both predator

species stayed motionless on the bottom of the container most of the time, sometimes for over one hour.

I counted an attack of a predator against the experimental tadpoles as one strike if it touched the bottom of the floating arena. To discriminate an attack from other rare behaviours in which predators touched the bottom of the arena (e.g. swimming to the water surface to swallow air), I defined the following behavioural sequences as an attack: (1) if a *Hoplobatrachus occipitalis* larvae swam from the ground of the container directly to a *P. microps* tadpole, turned on its back and touched with an open mouth the transparent base of the floating arena that physically separated the predator from the experimental tadpole. After the unsuccessful attack the predatory tadpole sank back to the bottom, (2) if a *P. microps* tadpole was detected, the killifish straightened up in a sloping position and swam very slowly towards the prey. Thereby, the predator compensated all movements of the tadpole by concomitant changes of direction during its approach until it touched the base of the arena below the tadpole.

At the end of each trial I obtained the number of strikes of the two predators against the 1 to 10 prey-tadpoles in different distribution patterns. The mean per capita strike rate was calculated by dividing this number by the number of tadpoles that were present in that trial. To test for statistical differences between numbers of strikes depending on different densities and distributions of tadpoles, data from treatments 1 to 5 were compacted and calculated by a one way repeated measure ANOVA (StatView 5.0 for Macintosh).

The *P. microps* tadpoles did not react to the predators' attacks. They were never injured and showed no signs of distress. In correspondence with the behaviour of this tadpole species in natural ponds, experimental tadpoles stayed motionless most of the time floating on the water surface of their compartment. From this position there was a distance of about 3 cm to the bottom of the container and therefore from the predator if it touched the base of the arena. Obviously, the visual recognition of a predator's attack alone seems insufficient to initiate a flight reaction in *P. microps* tadpoles.

At the end of the experiments all tadpoles and fishes were returned to their natural pools.

RESULTS

In control trials (treatment 6) where no tadpoles were placed in the arena, no predator attacks were recorded. These trials, which were meant to serve as a baseline, were thus omitted from the analysis.

The total number of strikes towards the arena differed between experimental treatments (ANOVA: $F_{4,48}=10.4$, $P<0.001$). Fischer's PLSD posthoc tests show that the total strike rate of predators towards tadpoles that were isolated in separate compartments increased with the total number of tadpoles in the arena (Fig. 1, Table 1). For aggregated tadpoles, however, no significant density effect was seen between the two treatments (Fig. 1, Table

TABLE 1. Results of the posthoc test for differences between numbers of strikes towards the arena in dependence on the different densities and distributions of tadpoles.

Fisher's PLSD: total strike rate	Mean. diff.	<i>P</i>
1 vs. 5 aggregated	-1.15	0.45
1 vs. 5 random	-2.85	0.06
1 vs. 10 aggregated	-2.15	0.16
1 vs. 10 random	-6.08	0.0002
5 random vs. 5 aggregated	1.69	0.27
5 random vs. 10 random	-3.23	0.04
5 aggregated vs. 10 aggregated	-1.00	0.51
10 random vs. 10 aggregated	3.92	0.01

1). Most notably, the posthoc test revealed that fewer attacks were directed towards the arena when ten tadpoles were clumped in one compartment than when ten tadpoles were randomly distributed in different compartments.

Analyzing the per capita strike rates (Fig. 2) by ANOVA, there was no statistical difference between the five treatments ($F_{4,48}=0.95$, $P=0.44$).

DISCUSSION

The present study demonstrates that larger groups are more frequently attacked by predators than smaller ones. This is consistent with the encounter effect where

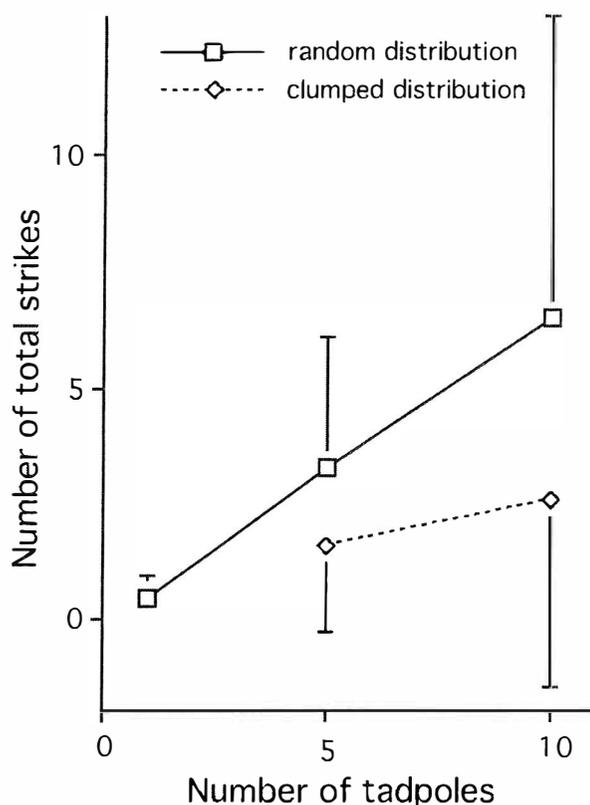


FIG. 1. Relationship between the mean number of total strikes of the predators towards the arena and the number of tadpoles present (either aggregated in one compartment or randomly distributed across the 40 compartments). Standard deviations are given as either positive or negative error bars. Each point represents the mean of 13 observations.

larger groups are more likely to be detected by a predator and are more attractive than smaller ones (Siegfried & Underhill, 1975; Watt & Chapman, 1998). The key factor seems not to be aggregation size *per se*, but the stronger movements in larger groups (Krause & Godin, 1995). Models that combine the encounter effect with the counteracting dilution effect indicate that protection against predators or parasites is provided when the probability of detection of a group does not increase in proportion to the increase in group size (Turner & Pitcher, 1986; Wrona & Dixon, 1991; Mooring & Hart, 1992). Evidence of such antagonistic relations between encounter and dilution effects is given for some species (Duncan & Vigne, 1979; Foster & Treherne, 1981; Cresswell, 1994).

In correspondence with the combined models, I found increasing total strike rates of predators with increasing density of prey tadpoles, but also a significant difference in total strike rate of predators between clumped and randomly distributed tadpoles at high equivalent densities (Fig. 1). Thus, highly aggregated tadpoles were less often attacked than the same number of randomly distributed tadpoles.

The risk of being preyed upon for an individual tadpole did not differ significantly between clumped and random distributions. However, the low statistical power warrants caution for this conclusion, and the ac-

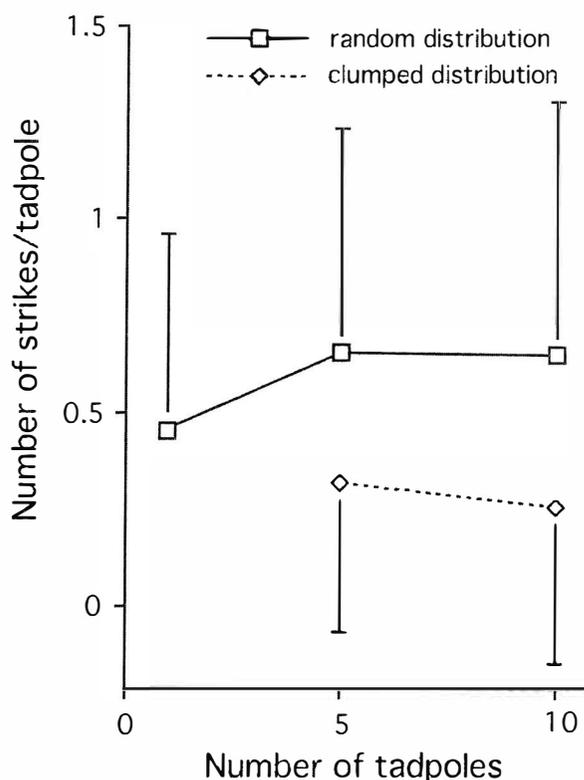


FIG. 2. Relationship between the mean number of per capita strikes of the predators towards the arena and the number of tadpoles present (either aggregated in one compartment or randomly distributed across the 40 compartments). Standard deviations are given as either positive or negative error bars. Each point represents the mean of 13 observations.

tual data (Fig. 2) suggest that more replicate trials might have detected benefits to the individual tadpole from aggregating.

The advantage for aggregated *P. microps* tadpoles is probably not simply a result of the dilution effect but, additionally, might be caused by the particular foraging behaviour of the predators. The hunting strategy of both the predators used can be classified as a sit-and-wait strategy. These predators mostly lay motionless on the base of the container and moved only occasionally and slowly through the water. The translucent tadpoles swimming on the water surface can only be detected by predators at a short distance. The probability of reaching such a distinct area in which a predator can detect one single tadpole or one dense aggregation depends on the intensity of the predator movements. For example, the chance for a sit-and-wait predator staying in a pond with two prey tadpoles to detect one of these tadpoles when they were randomly distributed is much higher than to detect these two tadpoles when they are in a group. By contrast, the chance for highly mobile predators to detect one of two randomly distributed tadpoles or these two tadpoles when they are in a group seems to be nearly the same. This applied especially if tadpole species are less conspicuous and if the attractiveness of an aggregation increased to a lesser extent than the increase in group size.

As most potential tadpole predators in the savanna ponds of the study area are sit-and-wait predators (Rödel, 1998), this hunting strategy provides an additional incentive for *P. microps* tadpoles to form large aggregations under high predation pressure. The importance of the hunting strategy of predator species on the survival rate of aggregated prey and thus on the constraints for prey to aggregate in the presence of predators with a defined foraging strategy has been pointed out in different studies (Parrish, 1993; Cresswell *et al.* 2003).

Furthermore, as the investigated predators of *P. microps* tadpoles visually orient towards – and attack – individual prey items, they may be subject to the confusion effect (Neill & Cullen, 1974). This probably leads to a further advantage of being aggregated in this species but was not quantified in this study. Likewise, it is to be expected that the Trafalgar effect plays an important role as an anti-predator strategy. This was indicated by the observed rapid synchronous flight reactions of *P. microps* tadpoles to dissolve aggregations once attacked by the predator *H. occipitalis* and by the ascertained sensitivity towards conspecific alarm substances (Rödel & Linsenmair, 1997).

ACKNOWLEDGMENTS

I thank T. U. Grafe, S. Böll, A. G. Nicieza, Rödel, M.-O. and one anonymous referee for valuable comments on an earlier draft of the manuscript and K. Mody for the identification of the killifish. This study was supported by the Volkswagen-Stiftung (AZ I/64 102). I

thank the Ministre des Eaux et Forests and the Ministre de la Recherche Scientifique, Republique de Côte d'Ivoire for granting the research permit for doing field work in the Parc National de la Comoé.

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Accepted: 1.10.04

DESCRIPTION OF A NEW *EUPSOPHUS* SPECIES (AMPHIBIA, LEPTODACTYLIDAE) FROM THE REMNANTS OF MAULINO FOREST, CENTRAL CHILE

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The description of a new species of *Eupsophus*, from Los Queules National Reserve (35° 59' S, 72° 41' W) adds a new endemic taxon to the fauna of telmatobines (Amphibia, Leptodactylidae) of Chile, and extends the geographical distribution of the genus approximately 200 km to the north. The morphological description of the new species includes a karyotype description and molecular data of the mtDNA 12S gene. Chromosome and molecular evidence supports the assignment of the new species to *Eupsophus*, allowing comparisons with congeneric and other lower telmatobine species. The diploid number of the new species is $2n=30$ and it presents the chromosomal XY system of chromosomal sex determination. It differs from *E. vertebralis* and *E. emiliopugini* (both $2n=28$) and groups with *E. calcaratus*, *E. contulmoensis*, *E. roseus*, *E. nahuelbutensis*, *E. insularis* and *E. migueli* ($2n=30$), the last two species also with morphologically differentiated XY chromosomes. This new endemic species reinforces the importance of Los Queules National Reserve as a unique reservoir of the biota of Maulino Forest, central Chile.

Key words: Anura, frog, taxonomy, karyotypes, mitochondrial DNA

INTRODUCTION

The Chilean amphibian fauna is not species-rich, but highly endemic, with 75% of the species restricted to the country (Formas, 1995). This fact might partially reflect the incomplete knowledge of Chilean biodiversity (Simonetti, 2001). In fact, new distributional records and new species of amphibians are described frequently, suggesting this fauna is yet to be fully assessed (e.g. Cuevas & Formas, 2002).

Ongoing sampling of fauna at the coastal Maulino forest of central Chile near Trehualemu, (including the Reserva Nacional Los Queules; 35°59'S -72°41' W; Fig. 1), nearby forest remnants, and the surrounding commercial pine plantations (*Pinus radiata*) have revealed several new taxa for the region, including over 20 species of epigeal coleopterans, three species of small mammals, one lizard and one snake previously unrecorded for this latitude. All these species were previously known to have their northernmost limit more than 200 km south of Reserva Nacional Los Queules (Grez, *et al.*, 2003; Rubio *et al.*, in press; Saavedra & Simonetti, 2000, 2001; Simonetti, 2001).

Among amphibians, we have recorded the presence of two species of leptodactylids, belonging to the genera *Eupsophus* and *Alsodes*. The presence of *Eupsophus* at Trehualemu represents a significant extension of the

distribution of the genus. The nearest record for an *Eupsophus* species is *E. roseus*, at Cordillera de Nahuelbuta (Veloso & Navarro, 1988; Nuñez *et al.*, 1999). Further examination of the specimens from Trehualemu, including karyotypic and molecular data, revealed that they belong to an hitherto undescribed species of *Eupsophus*. Here, we describe this new taxon.

MATERIALS AND METHODS

Twelve specimens of the new species were collected at night, deposited at the Departamento de Biología

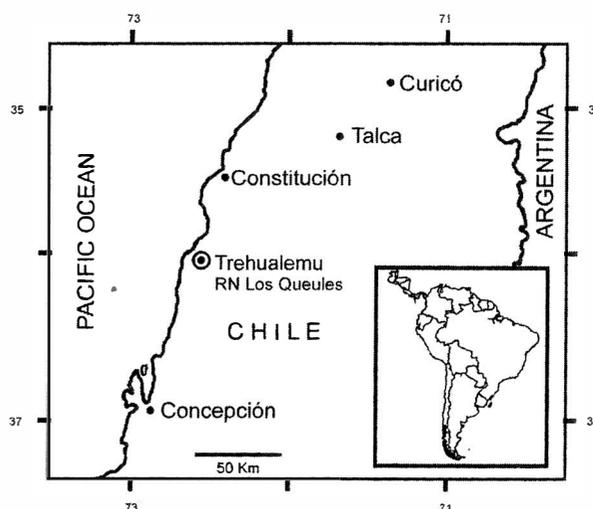


FIG. 1. Map of the type locality of *Eupsophus queulensis*. RN denotes Reserva Nacional Los Queules.

Celular y Genética, Universidad de Chile (DBGUCH), and later compared to congeneric species (*E. roseus*, *E. calcaratus*, *E. migueli*, *E. nahuelbutensis*, *E. insularis*, *E. contulmoensis*, *E. vertebralis* and *E. emiliopugini*) with regard to external morphology, patterns of coloration and karyotypes (Núñez, 2003). In adult specimens of both sexes and unsexed juveniles the following measurements were taken: snout-vent length (SVL), head width at the mid-tympanic level (HW), head length (HL), from the snout tip to posterior margin of the tympanic membrane, tympanic diameter (TD), distance between the anterior edge of the eye opening to the posterior edge of the nostrils (ON), eye diameter (ED), shortest distance between the eyes at the upper part of the head (IOD), tibia length (TL), distance between nostrils (IN), distance from the nostrils to the tip of the snout (NH). All measurements were taken with a digital caliper to the nearest 0.01 mm. The holotype and type series were subsequently transferred to the Museo Nacional de Historia Natural in Santiago (MNHNC), in accordance with Chilean regulations.

CHROMOSOMAL MARKERS

Two adult specimens, male MNHNC 3790 and female MNHNC 3793, were injected with colchicine 1%. Chromosomes were obtained from the intestinal epithelium, cornea and testicles. Intestinal and testicle tissues were subjected to a hypotonic treatment with distilled water and fixed in acetic acid 50%. Squash was performed after fixation. Cornea preparations were obtained by subjecting the eyes to hypotonic treatment with distilled water, and then fixing them in vapours of 100% of acetic acid (Bogart, 1973). The identification of homologous chromosomes was carried out determining the position of the centromeres and measuring the chromosomes with Micromesurer 3.3 (Reeves & Tear, 2000). Chromosome terminology follows Levan *et al.*, (1964).

MOLECULAR MARKERS

Sequence of the 12S gene of mtDNA of *Eupsophus* sp. was compared to putative congeners *Eupsophus vertebralis* (DBGUCH 3342), *E. calcaratus* (DBGUCH 2904) and other closely related species of lower telmatobines (Leptodactylidae): *Batrachyla taeniata* (DBGUCH 2943), *Telmatobius marmoratus* (DBGUCH 3384) and *Caudiverbera caudiverbera* (DBGUCH 3028). DNA was extracted using the phenol-chloroform method (Sambrook *et al.*, 1989). Primers L1091 and H1478 were used for PCR amplifications, after Kocher *et al.*, (1989). PCR products were purified using QIAgen and sequenced in ABI Prism 3100. Sequences were revised with CHROMAS (McCarthy, 1998) and BIOEDIT (Hall, 1999). All sequences were submitted to GenBank (accession numbers AY578817 - AY578822).

Phylogenetic relationships were inferred through PAUP*4.0b8a (Swofford, 2002) and MEGA (Molecular Evolutionary Genetic Analysis, version 1.2; Kumar *et al.*, 2001). Analyses were carried out using Maximum Parsimony (MP) and Neighbour Joining (NJ), using

Kimura two-parameter distances (Kimura, 1980). Internal support for each node was evaluated through bootstrapping (1,000 replicates).

RESULTS

DESCRIPTION OF A NEW SPECIES OF *EUPSOPHUS*

Eupsophus queulensis, new species

Ranita de Los Queules

Los Queules frog

Holotype. MNHNC 3798, adult male, collected by A. Veloso and K. Busse, on November 2003, at the east edge of Reserva Nacional Los Queules, 71 km W Cauquenes, coastal mountain range of the Costa, VII Maule Region (35° 59' S, 72° 41' W) at 450 m elevation, Chile (Fig. 2-3).

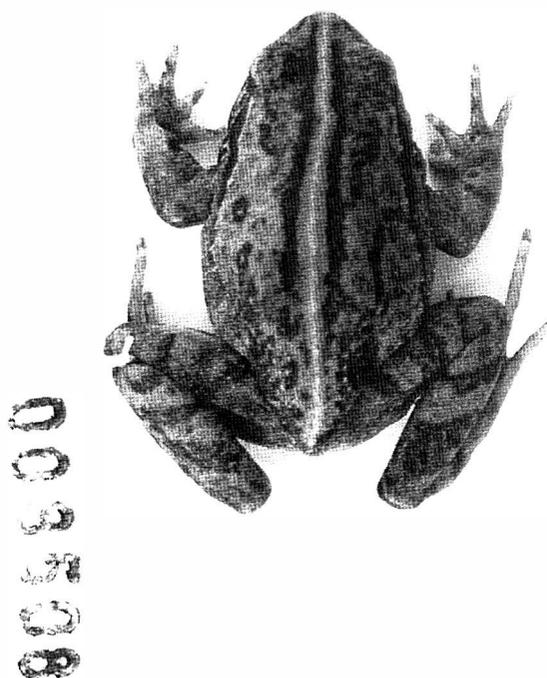


FIG. 2. Holotype of *Eupsophus queulensis*. Male MNHNC 3798.

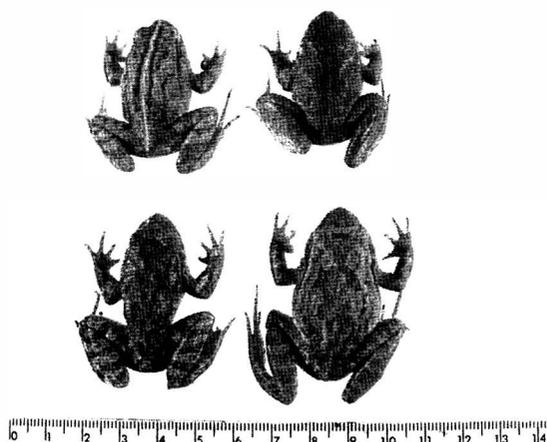


FIG. 3. Variations in the coloration pattern of *Eupsophus queulensis*.

TABLE 1. *Eupsophus queulensis* sp. nov. Morphometric data of the type series (mean, SD and range), and morphometric data of females and juveniles (all data in mm; see codes in methods).

Character	Adult range	Holotype	Paratypes						Mean±SD	Females		Mean±SD	Juveniles			Mean±SD
			3798	3787	3788	3789	3790	3791		3792	3793		3794	3795	3796	
SVL	34.00-47.30	39.8	37.20	37.85	40.60	34.00	41.65	39.70	38.69±2.57	45.50	47.30	46.40±1.27	26.60	25.70	29.50	27.27±1.99
HW	14.70-18.60	15.75	14.70	16.00	16.15	15.25	17.00	15.75	15.80±0.72	18.40	18.60	18.50±0.14	10.45	9.45	11.60	10.50±1.08
HL	13.30-16.70	15.40	14.65	13.30	14.60	15.10	13.5	15.35	14.56±0.85	16.25	16.70	16.48±0.32	8.45	7.45	7.9	957.95±0.50
TD	2.05-3.00	2.45	2.25	2.05	2.60	2.30	2.15	3.00	2.40±0.32	2.25	2.50	2.38±0.18	1.20	1.30	1.35	1.28±0.08
ON	2.10-2.95	2.30	2.95	2.50	2.20	2.10	2.25	2.50	2.40±0.28	2.65	2.55	2.60±0.07	1.75	1.20	1.90	1.62±0.37
ED	4.80-6.56	5.50	5.20	4.55	5.40	4.80	5.40	6.35	5.31±0.57	6.20	6.56	6.38±0.25	4.75	3.45	4.4	04.20±0.67
IOD	3.45-4.50	3.85	4.05	3.70	3.90	4.00	3.45	4.25	3.89±0.26	4.44	4.50	4.47±0.04	2.95	2.70	2.95	2.87±0.14
TL	20.55-25.05	20.60	20.80	20.65	20.55	21.60	22.55	21.90	21.24±0.79	25.05	25.05	25.05±0.00	14.35	15.60	16.75	15.57±1.20
IN	3.50-4.70	3.70	3.70	3.85	4.00	3.50	4.65	4.15	3.94±0.38	4.70	4.70	4.70±0.00	2.65	2.30	2.90	2.62±0.30
NH	2.51-4.10	3.45	3.45	2.51	3.15	3.10	4.10	3.20	3.28±0.48	3.40	3.70	3.55±0.21	1.70	1.65	2.1	51.83±0.28

TABLE 2. Comparative chromosomes types in species of *Eupsophus* genus except undescribed *E. nahuelbutensis* karyotype ($2n=30$): $2n$ = diploid number, NF = number of arms, m = metacentric, sm = submetacentric, st = subtelocentric, t = telocentric (Levan *et al.*, 1964). 14^* heterochromosome; $**$ Chromosomes with secondary constriction (nucleolar pair).

Species	Pair															$2n$	NF
	1	2	3	4	5	6	7	8	9	10	11	12	13	14*	15		
<i>E. vertebralis</i>	m	st	m	st	st	st	sm	m	m	m	m	m	t	m		28	56
<i>E. emiliopugini</i>	m	st	m	st	st	st	sm	m	m	m	m	m	m	m		28	56
<i>E. insularis</i>	m	sm	st**	m	t	m	t	t	m	t	m	t	t	m/t*	t	30	44 / 45
<i>E. calcaratus</i>	m	sm	st**	t	m	t	m	m	t	m	t	t	m	t	t	30	46
<i>E. migueli</i>	m	sm**	st	t	t	t	m	m	m	t	t	m	t	m/t*	t	30	44 / 45
<i>E. roseus</i>	m	sm**	st	t	t	t	m	m	m	t	t	m	t	m/m*	t	30	46
<i>E. contulmoensis</i>	m	st	st**	t	t	m	m	t	t	m	m	t	t	m	t	30	46
<i>E. queulensis</i> sp. nov.	m	st	st	t**	t	m	t	m	sm	t	t	m	t	m/t*	t	30	44 / 45

Paratypes. Males MNHNC 3787-3792, females MNHNC 3793-3794, and unsexed juveniles MNHNC 3795-3797, collected by J. L. Celis-Diez and P. C. Guerrero, on September 2002, at the type locality.

Diagnosis. *Eupsophus queulensis* is a medium-sized frog, distinguished by: (1) truncate snout, (2) left epicoracoid superimposed to the right one, (3) xiphisternum without a notch, (4) prominent external and internal carpal tubercles, (5) yellow iris in life, (6) two melanic dots on both sides in the posterior half of the dorsal region, (7) reddish ventral surfaces, (8) an heteromorphic pair of sexual chromosomes, with a Y metacentric chromosome, and (9) secondary constriction at both homologs of the largest telocentric pair of chromosomes.

Description of the holotype. Adult male of medium size, head as long as broad (HL/HW = 0.98), slightly more than 1/3 of the SVL (HL/SVL = 0.38), laterally spaced narines, canthus rostralis almost flat, short and truncate snout, NH/ON = 1.35, prominent tympanic annulus, tympanic membrane covered with skin, vomerine teeth separated in the half line behind the lateral border of the elliptic coanes, robust forearms, nuptial pads on first finger. Hind limbs slender, tibio-tarsal articulation surpasses the anterior border of the eye orbit when hind limb is flexed toward the head. Relative length of toes: 4 > 3 > 5 > 2 > 1. Inner metatarsal tubercle present, outer metatarsal tubercle absent, interdigital membrane absent. Relative length of fingers: 3 > 1 > 4 = 2. Inner and external carpal tubercles present. Left epicoracoides superimposed to the right one, xiphisternum without notch, smooth dorsal and ventral skin, and a cutaneous tarso-metatarsal fold present, smooth plantar and palmar surfaces. Measurements are summarized in Table 1.

Coloration in alcohol. Small gray dots along the edge of the upper jaw. Gray dorsal surface with dark stripes, extending from behind the eyes to the dorsum, on both sides of the mid-line. A conspicuous white mid-dorsal strip, extending from the tip of the snout to the vent. Posterior half of the dorsum with irregular black spots, forming a geometric figure on both side of the mid-line. Two symmetrical black spots in the dorso-lumbar area. Postorbital black spots extending laterally to the proximal end of the arms. Ventral pale yellow with black pigmentation in the throat region. Anterior extremities with small black spots. Posterior limbs with transverse black lines.

Coloration in life. The coloration in life contrasts with that of the preserved animals. Dark spots are more

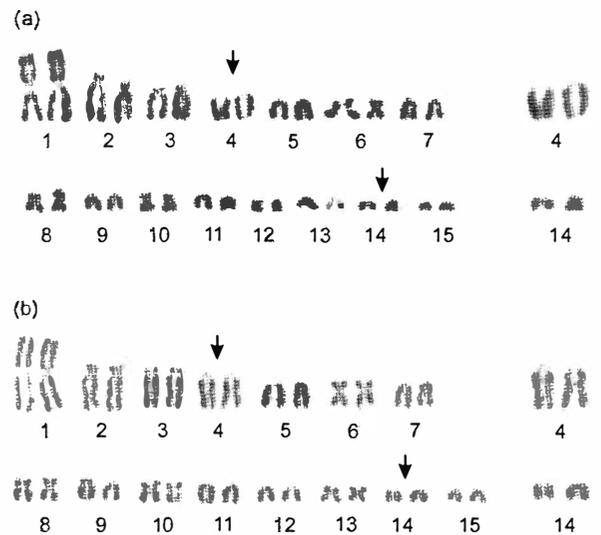


FIG. 4. Male (a) and female (b) karyotypes of *E. queulensis*. Arrows indicate the sexual chromosomes and the nucleolar chromosome pair. In the frames, these chromosomal markers are identified.

defined and the gray coloration presents a reddish tinge. Also, the iris of the live animal has a yellow upper part and brown lower part. The eyelid edge is blue-gray.

Variation in the coloration pattern. Other male and female specimens do not show a yellowish dorsal mid-line (Fig. 3).

Karyotype. Diploid number is $2n=30$ with a fundamental number (NF) of 45 in males and 44 in females (Table 2, Fig. 4). The sexual pair is heteromorphic with a metacentric Y chromosome. The largest pair of telocentric chromosomes is the nucleolar pair, depicting a secondary pericentromeric constriction.

Molecular markers. A mtDNA 12S fragment of 319 base pairs was amplified. Maximum Parsimony (MP) and Neighbour Joining analyses gave the same tree topology. MP resulted in a single tree of 70 steps, with a Consistency Index of 0.86 and a Retention Index of 0.66. In this tree, *E. queulensis* shares a clade with *E. calcaratus* and *E. vertebralis*, apart from species of different genera. Within this clade, *E. queulensis* is the sister species of *E. calcaratus*, with *E. vertebralis* as the sister taxon of that clade (Table 3, Fig. 5).

Natural history. Field observations were carried out at the Reserva Nacional Los Queules, nearby forest fragments and surrounding pine plantations. Within native Maulino forest, individuals were found only in moist habitats including shallow streams, soil cavities, leaf lit-

TABLE 3. Genetic distances among species studied. In bold are the averaged distances estimated using Kimura 2 parameter model (below diagonal) and in italics is the standard error estimated by 1000 bootstrap replicates (above diagonal).

Species	1	2	3	4	5	6
1. <i>Batrachyla taeniata</i>		<i>0.022</i>	<i>0.020</i>	<i>0.019</i>	<i>0.017</i>	<i>0.015</i>
2. <i>Caudiverbera caudiverbera</i>	0.136		<i>0.026</i>	<i>0.027</i>	<i>0.024</i>	<i>0.019</i>
3. <i>Eupsophus calcaratus</i>	0.111	0.178		<i>0.012</i>	<i>0.016</i>	<i>0.019</i>
4. <i>Eupsophus queulensis</i>	0.096	0.182	0.044		<i>0.015</i>	<i>0.019</i>
5. <i>Eupsophus vertebralis</i>	0.087	0.156	0.072	0.068		<i>0.015</i>
6. <i>Telmatobius marmoratus</i>	0.072	0.112	0.103	0.102	0.065	

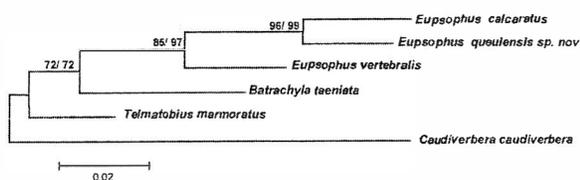


FIG. 5. Maximum Parsimony tree based on 12S mtDNA sequences for telmatobine species. The numbers indicate bootstrap values for the groupings. Bootstrap values are indicated on each node of the tree (MP/ML respectively) after 1000 pseudoreplicates.

ter and under logs. No individual was recorded in surrounding pine plantations. Vegetation in the Maulino forest is dominated by a deciduous tree, *Nothofagus glauca*, along with evergreen trees such as *Persea lingue*, *Gevuina avellana*, *Aetoxicum punctatum* among others (see Bustamante *et al.*, in press). Vocalizations, composed of a low frequency sound followed by an abruptly finishing crescendo, were heard from September to January. Male and female pairs were found together with egg masses or recently hatched embryos in soil cavities near streams. Also, larvae at stage 35-36 (Gosner, 1960) were found in shallow waters. Eggs have large amounts of yolk; neither eggs nor larvae exhibit pigmentation. In gravid females, eggs are visible through the skin at the lower part of the abdomen. These features are shared with other *Eupsophus* species (Díaz, 1986).

Geographic distribution. *Eupsophus queulensis* is known only from the type locality.

Etymology. The new species is named after the Reserva Nacional Los Queules, one of the few protected areas aiming to conserve the unique biota of the coastal Maulino forest.

DISCUSSION

Eupsophus is an endemic genus which comprises eight species: *E. calcaratus*, *E. contulmoensis*, *E. emiliopugini*, *E. insularis*, *E. migueli*, *E. nahuelbutensis*, *E. roseus* and *E. vertebralis*. As such, it is the most diversified genus of leptodactylids in the *Nothofagus* forests (Núñez, 2003). Some species have their geographical range restricted to their type locality, e.g., *E. contulmoensis* (Monumento Nacional Contulmo, Cordillera de Nahuelbuta), *E. nahuelbutensis* (Parque Nacional Nahuelbuta, Cordillera de Nahuelbuta) and *E. insularis* (Isla Mocha; Formas 1995). Others have broader distributions, and exhibit geographic replacement with latitude, such as *E. roseus* by *E. calcaratus* and *E. vertebralis* by *E. emiliopugini* (Núñez *et al.*, 1999). The geographic distribution of *E. queulensis* ought to be assessed by sampling beyond the Trehualemu area.

Eupsophus is characterized by medium-sized species, eggs and larvae without pigmentation and polymorphism in colour pattern. Morphologically, features that distinguish *E. queulensis* from *E. roseus* are a left epicoracoid superimposed to the right in the former,

while in *E. roseus* the opposite is true: the right being superimposed to the left. Iris colour in *E. queulensis* is yellow in the upper part and brown in the lower part, whereas it is orange in *E. roseus*. It differs from *E. calcaratus* by its truncate snout. It also differs from *E. insularis* in dorsal coloration, which is brown with irregular yellow spots as compared to the dark spots found in *E. queulensis*. The new species differs from *E. migueli* in dorsal coloration, in its two conspicuous spots at both sides of the half line in the lumbar region, and in presenting an unnotched xiphisternum. *Eupsophus queulensis* differs from *E. nahuelbutensis* in the colour pattern of the ventral region, which is whitish with dark-brown pigmentation in *E. nahuelbutensis* (Núñez, 2003). Finally, it differs from *E. contulmoensis* in presenting prominent external and internal carpal tubercles. Also, the ventral coloration of this last species is brilliant yellow (Formas, 1992).

Karyotypically, *Eupsophus* species can be grouped according to their diploid number (Formas, 1980; Iturra & Veloso, 1989; Formas, 1991; Cuevas & Formas, 1996, Núñez 2003). A first group is comprised by *E. emiliopugini* and *E. vertebralis* ($2n=28$) and a second one includes *E. calcaratus*, *E. contulmoensis*, *E. migueli*, *E. roseus* and *E. nahuelbutensis* ($2n=30$; Díaz & Veloso, 1979; Formas, 1991, 1992; Núñez, 2003), to which *E. queulensis* should be added. *Eupsophus queulensis* differs from its congeneric species in the location of the secondary constriction (Table 2). Three of these (*E. insularis*, *E. migueli* and *E. queulensis*) have sexual heteromorphic chromosomes in the same stage of differentiation, with an X telocentric and a Y metacentric chromosome, the male being the heterogametic sex. *Eupsophus roseus* also has a pair of sex chromosomes but with the same morphology (X and Y metacentrics) that can only be determined by banding techniques (Iturra & Veloso, 1989). Despite having the same $2n=30$ and sex chromosomes, *E. queulensis* differs from *E. migueli* because the former has a telocentric shape of the nucleolar pair (AgAsNOR) while in *E. migueli* is sub-telocentric.

Based on external morphology, karyotype and molecular data, the specimens collected at Trehualemu can be assigned to *Eupsophus*. Despite the reduced number of species evaluated, phylogenetic analysis of mitochondrial DNA confirms that the new species described here belongs to the genus *Eupsophus*; within this genus *E. queulensis* is closer to *E. calcaratus* than to *E. vertebralis*. High bootstrap values support this assertion (Fig. 5). Similarly, the differentiation of a pair of sexual chromosomes, shared with *E. insularis* and *E. migueli*, suggests a narrow phylogenetic relationship with these two species whose chromosomal system of sex determination (XY-type) is in the same stage of differentiation (Iturra & Veloso, 1989; Cuevas & Formas, 1996).

The Maulino coastal forest contains a high number of endemic and threatened species such as *Gomortega keule* (Gomortegaceae), *Pitavia punctata* (Rutaceae) and *Berberidopsis corallina* (Berberidopsidaceae)

among plants, *Dromiciops gliroides* (Microbiotheriidae), *Oncifelis guigna* (Felidae), *Pudupudu* (Cervidae) among mammals, and *Megalometides discors* (Curculionidae) among insects. This unique biota is protected in the Reserva Nacional Los Queules.

The finding of more southern species, typical of the Valdivian forest, such as *D. gliroides*, *Geoxus valdivianus* and *Irenomis tarsalis* (Muridae), *Tachymenis chilensis chilensis* (Colubridae), *Liolaemus cyanogaster cyanogaster* (Iguanidae), *Protosphindus bellus* (Sphindidae) and *Pteroderes tuberosa* (Ulodidae) along with a new species of *Eupsophus*, previously regarded as a southern genus, suggests that the Maulino forest is the current northernmost limit of the Valdivian fauna, and also reinforces the importance of this reserve for the conservation of this unique biota (Simonetti, 2001; Saavedra & Simonetti, 2001; Grez et al., 2003). In Trehualemu, pine plantations are the dominant landscape feature, while native forest is restricted to small scattered remnants. The 145 ha Reserva Nacional Los Queules is one of the protected areas aiming to conserve the Maulino forest, which faces a high rate of destruction and replacement (Grez et al., 1997). The absence of *E. queulensis* from the extensive pine plantations suggest that, if restricted to native forest, *E. queulensis* could be threatened due to the scanty surface of native forest remnants.

ACKNOWLEDGEMENTS

We are grateful to Forestal Terranova S.A. and to Corporación Nacional Forestal (CONAF, Chile), for allowing us to work on their properties. Thanks are due to K. Busse for improving our field observations, F. Campos for his guide in the field and to C. Correa, N. Vergara, P. Bachmann and M. Torres for their collaboration in the laboratory. M. Yarrow improved the English translation. Field work for this research was partially supported by DID, Universidad de Chile to A. Veloso and FONDECYT 1010852 to J. A. Simonetti. J. L. Celis-Diez is a Graduate Fellow from CONICYT, Chile.

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Accepted: 1.10.04

VARIATION IN ANURAN ABUNDANCE ALONG THE STREAMS OF THE WESTERN GHATS, INDIA

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In order to test the hypothesis that anuran abundance does not vary between forest and commercial plantations, anurans were sampled along the streams of reserve forests, and adjoining cardamom and coffee plantations on the western slopes of the Western Ghats. The species composition and relative abundance varied significantly between the three habitats. A few species showed complete dependence on the forest whereas many common species were least affected by habitat alterations associated with plantations. Analysis of variance revealed significant differences in the abundance of *R. temporalis*, *Micrixalus saxicolus*, *Fejervarya limnocharis*, *Euphlyctis cyanophlyctis* and *Bufo melanostictus*, whereas the difference was not significant for *Indirana beddomii*, *Nyctibatrachus* species and *Polypedates* species.

Key words: amphibian, frog, species composition, plantations, stream transects

INTRODUCTION

Amphibian population declines have been reported from various parts of the world (Richards *et al.*, 1993; Fisher & Shaffer, 1996; Lips, 1998; 1999). One of the main reasons for such declines has been found to be habitat modification (Daniels, 1991; Lips, 1998; Lips *et al.*, 2003). Anthropogenic activities such as deforestation (Petranka *et al.*, 1993) have been highlighted as an important threat. Amphibians relying on specific microhabitat features may be in danger of declining because of extensive forestry-related disturbances. In southern India, selective logging in the primary forests has converted the original forests to fragmented secondary forests. Such isolation and fragmentation of forests results in drying of the forest floor and altered vegetation composition. Because amphibians show site fidelity and have limited dispersal capabilities, disturbances such as fragmentation, road construction and cutting of trees may disrupt metapopulation systems (Osawa & Katsuno, 2001) and cause local extinction of species (Blaustein *et al.*, 1994).

The forests of the Western Ghats are considered to be one of the world's biodiversity hotspots, with endemic plants, butterflies, birds and amphibian species. Until now 196 species of anurans have been reported from India (Daniel, 2002) and approximately 75% of Indian amphibians are endemic to the Western Ghats (Oommen *et al.*, 2000). The distributions of amphibians are uneven even across India and within the Western Ghats. The medium elevations are considered to be the richest zone in the Ghats (Daniels, 1992). Most of the ecological works on Indian anurans are restricted to distributional records. The demographic status of anurans in such pristine forests, where there is a concentration of many species, remains anecdotal. Encroachment upon

wilderness areas by major anthropogenic activities and habitat modification for establishing new plantations such as coffee and cardamom, diversion of hill streams, and modification of water bodies for farming have resulted in loss of breeding sites in recent years. The synergistic interactions among diverse human activities such as the land use change, climatic changes, habitat degradation, vegetation removal and illegal removal of non timber forest produce (NTFP) has made the conservation of the forest and wildlife a major challenge in these forests.

In the context of global amphibian population decline, it is important to know the situation in one of the world's amphibian diversity hotspots, the Western Ghats. Many researchers have used observational studies and sometimes combined them with historic records to document amphibian declines. However, in areas where historic data do not exist, understanding the population status has become impossible. Regular long-term monitoring of the amphibians in this sensitive area is crucial to plan conservation strategies and management activities to save species from extinction. Our presence/absence survey in one such locality within the area showed the decline in calling frogs during the early monsoon season, especially *Hoplobatrachus tigerinus* during 1999-2000 (personal observations, S.K.N.). Elsewhere the reasons for *Hoplobatrachus tigerina* declines in paddy fields are thought to be due to the indiscriminate use of chemicals in agricultural lands and trading of frog's legs (Dutta & Hejmadi, 1981). The Conservation Assessment and Management Plan for Amphibians of India used the IUCN criteria for status assessment and has already reported the species as vulnerable (Molur & Walker, 1998). The amphibian species preferring cool evergreen forest habitats have patchy distributions, probably due to habitat destruction and fragmentation (Daniels, 1992).

We sampled the streams for anurans in 2001 and 2002, from January to May, when the streams flow is

less swift and sampling was possible in the difficult sloping terrain of the Western Ghats. There are no pre-existing data on anuran abundance from unmodified habitats that the present data could be compared to. Therefore, only horizontal habitat comparisons can be done to assess the effect of habitat modification on anuran abundance. Here, we report for the first time the abundance of anurans along streams in the forests of the Western Ghats, and provide a measure of comparative species composition and abundance in the two adjoining types of plantations. The objectives of our study were to (1) to establish baseline data on the relative abundance of a few selected riparian species in the pristine forest streams so that the demographic assessments can be done on the basis of present status of anurans for future population monitoring programme; and (2) to understand the effect of habitat modification on the abundance of such species, as it is important for habitat conservation and management implementations.

METHODS

Bisale reserve forest (12° 15'N and 75° 33'E) is managed by the Karnataka State Forest Department. The forest is wet evergreen secondary forest. Three different sites were selected for the study, one undisturbed forest and two modified habitats (plantations). Care was taken while selecting the plantation so that they were all located adjacent to each other, so that elevation and other physical parameters remained almost same. The forest site was at a higher elevation on the crest of the western slopes of the Western Ghats at an elevation of 840 m above sea level. The Western Ghats in this area receive an average of 5500 mm rainfall annually from the south-west monsoon rains. This in turn had turned the Western Ghats into a main watershed for the Indian Peninsula, with many streams and rivers. Many hill streams in the study area feed the west flowing river Kumaradhara that in turn joins Arabian Sea. The vegetation is semi-evergreen (Champion & Seth, 1968) and classified as *Dipterocarpus indicus* – *Kingiodendron pinnatum*–*Humboldtia brunonis* forest type (Pascal, 1988). The edges of reserve forests in this region are circled by numerous small private land holdings. These private lands, which were once forested, are being modified for the cultivation of commercial crops, paddy fields and plan-

tations. The main large-scale plantations in the area are cardamom and coffee estates.

The second site was in Hosagadde, a cardamom estate (28° slope) located on the hill ranging from 840-920 m elevation, adjoining the Bisale Reserve Forest on the eastern side. The annual average temperature during 2002 was recorded to be 21.11° C, the humidity was 62.89% and annual total rainfall was 4790 mm. The cardamom (*Elettaria cardamomum*) is grown on the cool slopes of hills where there is heavy rainfall and more moisture, whereas coffee requires less rain and drier weather to produce seeds. The cardamom plantations in these areas were grown under the canopy, without clearing the undergrowth. Neither fertilizer nor insecticides were sprayed.

The third site was a coffee plantation, 3 km to the north-east of the forest site. Although the coffee plantations were developed on contours, the general terrain was less undulating when compared to cardamom and forested sites (Table 1). However, coffee (*Coffea arabica*) plants were grown in a completely modified habitat. Coffee plantations need more open canopy and clear ground. Hence the canopy cover is removed off on a yearly basis and the undergrowth cleared. Coffee plants were fertilized every year and the spraying of insecticide was used as part of the cultivation practice.

Streams were treated as special transects (Inger & Colwell, 1977) and were sampled for the occurrence of anurans. The data were collected from early morning transects (0530 to 0700 hr) along fixed segments (500-820 m) of the streams and sampled repeatedly. The streams selected were flowing in curves and covered the entire habitat of our interest. Therefore, the stream surveyed was representative of the entire riparian habitat of the areas in forest and plantations. Five volunteers, one in the middle of the stream, two on either sides very close to the edge of the stream and two on the shores, moved forward and made a systematic search of the area. We monitored all the stream transects by scanning the banks and marginal vegetation after flushing out frogs by sticks, scanning emergent rocks, uprooting the stones, sticks and litters within the streams. Care was also taken to release the captured frogs well behind the survey line so that recounting of the same individual was avoided. We recorded data from 820 m segments of the

TABLE 1. Site characteristics for the three habitats studied.

Site characteristics	RF Stream	Cardamom stream	Coffee stream
Elevation	< 850 m	920-840 m	900 m
Topography	Hilly	Hilly	Flat
Vegetation	Secondary forest	Plantation	Plantation
Canopy cover	Covered	Covered	Clear
Streams: Width (m)	4–5.8 m	4–7 m	6–9 m
Gradient/Clarity	Steep/clear	Steep/clear	gentle/clear
Bottom	Rocky	Rocky	Rocky & few mud bottom pools

stream in the forest and cardamom habitats and sampled them 13 and 24 times respectively. In the coffee estate, we marked a 500 m stream segment and sampled five times (Inger & Voris, 1993). Stream width varied from 6.1 to 10.2 m and the mid-stream depth varied from 0.7 to 1.3 m. Intervals between the sampling periods varied from five to eight days.

As the transect length varied between habitats, abundance was measured as the total number of frogs observed per 500 m transect. Anuran abundance variations along the streams running in three different habitats were compared using chi-square tests. Within-species comparisons of abundance in three different habitats were done using multiple comparisons available in one-way ANOVA. Tukey HSD post-hoc tests were used to compare the relative abundance of each species between the habitats to identify the homogenous habitats ($\alpha = 0.05$).

RESULTS

Eight common species representing three families (Ranidae, Bufonidae and Rhacophoridae) accounted for more than 90% of frogs observed along the streams at forest sites. The rare sightings included a single individual of *Ansonia ornata* within the stream of forests. The streams of cardamom estate and forest showed equivalent species composition. However, the stream transects in coffee habitat revealed only six anuran species. Anuran abundance varied between the three habitats ($\chi^2=1882.1$, $df=16$, $P=0.001$). They showed significantly varied patterns of distribution in all the three different habitats (Table 2). Few species showed complete dependence on the forest streams. However many common species were least affected by habitat al-

terations caused by plantations. The ANOVAs revealed significant differences in mean abundance in four species, but the difference was not significant in *Indirana beddomii* ($F_{2,39}=1.07$, $P=0.352$), *Nyctibatrachus* sp. ($F_{2,39}=1.64$, $P=0.205$) and *Polypedates* sp. ($F_{2,39}=0.82$, $P=0.446$). The cardamom estate and the forest formed a single group for five species and due to the significant variation in their abundance, the coffee estate was singled out from the group. But the abundance of three species did not significantly vary between the habitats (Table 2 and Table 3).

INDIVIDUAL SPECIES ACCOUNTS

Rana temporalis. This is a medium sized, bronze coloured, nocturnal frog. They are active both day and night (Daniel, 2002) and retreat into rocky or mud crevices in the banks of streams. Sometimes we have observed them basking on the rock outcrops of streams. The species has long legs and leaps over considerable distances when disturbed. The juveniles are always found to inhabit the sandy banks close to water. We never sighted them away from the water source.

Rana temporalis showed an uneven pattern of distribution, with 75% abundance in the coffee estate stream, but only 12.3% and 25.3% abundance along forest and cardamom streams, respectively. The mean number of frogs per transect was significantly higher in the coffee and cardamom estate streams than in the forest ($F_{2,39}=449.98$, $P=0.0001$). Although the variation in mean numbers of *R. temporalis* in the streams of forest and cardamom estates was significant and formed a common subset, their abundance was extremely high in the streams of the coffee estate and therefore that habitat formed a different subset (Table 2, 3).

TABLE 2. Mean numbers and *F*-values for anuran abundance in forests and two adjoining plantations of Bisale Reserve forests. Ranges are given at 95% confidence interval for Mean. *N*=number of sampling episodes.

Species	Reserve Forest stream Mean \pm SE Range (<i>N</i> =13)	Cardamom stream Mean \pm SE Range (<i>N</i> =24)	Coffee stream Mean \pm SE Range (<i>N</i> =5)	<i>F</i>	<i>P</i>
<i>Rana temporalis</i>	6.03 \pm 1.7 (2.23-9.918)	23.9 \pm 3.9 (15.83-31.99)	271.2 \pm 13.19 (234.56-307.83)	449.9	0.001
<i>Bufo melanostictus</i> juv	0	0.33 \pm 0.15	4.6 \pm 0.4 (3.48-5.71)	100.9	0.001
<i>Micrixalus saxicolus</i>	19.69 \pm 3.57 (11.89-27.49)	22.79 \pm 2.38 (17.86-27.72)	0	8.18	0.001
<i>Fejervarya limnocharis</i>	2.53 \pm 0.74 (0.9-4.1)	3.95 \pm 0.65 (2.59-5.31)	30.2 \pm 4.22 (18.47-41.92)	90.4	0.001
<i>Indirana beddomii</i>	9.53 \pm 2.50 (4.08-14.99)	15.25 \pm 2.91 (9.22-21.27)	16.2 \pm 1.3 (12.43-19.96)	51.07	0.332
<i>Nyctibatrachus</i> sp.	8.61 \pm 2.00 (4.24 -12.99)	27.66 \pm 8.04 (11.02-44.30)	19.20 \pm 0.96 (16.50-21.89)	1.64	0.205
<i>Euphlyctis cyanophlyctis</i>	0	0.12	3.20 \pm 0.8 (0.97-5.42)	48.09	0.001
<i>Polypedates</i> sp. juv.	0.46 \pm 0.26 (0.12-1.04)	0.62 \pm 0.22 (0.16-1.08)	0	0.82	0.44

TABLE 3. Post-hoc Tukey HSD test showing homogeneous habitats. Means for group in homogeneous subsets are displayed for three habitats, R.F., species along the stream of Reserve forest, Cardamom, species along the stream of Cardamom estate; Coffee, species along the streams of the coffee plantation.

Species	Subset for $\alpha=0.05$			
		1		2
<i>Rana temporalis</i>	R. F.	6.07	Coffee	271.2
	Cardamom.	23.91		
<i>Bufo melanostictus</i> juv.	R. F.	0.0	Coffee	4.6
	Cardamom	0.3		
<i>Micrixalus saxicolus</i>	Coffee	0.0	R. F	19.69
			Cardamom	22.79
<i>Fejervarya limnocharis</i>	R. F.	2.53	Coffee	30.2
	Cardamom	3.95		
<i>Euphlyctis cyanophlyctis</i>	R. F.	0.0	Coffee	3.2
	Cardamom	0.12		
<i>Indirana beddomii</i>	R. F.	9.53		
	Cardamom	15.25		
	Coffee	6.2		
<i>Nyctibatrachus</i> sp.	R. F.	8.61		
	Cardamom	19.2		
	Coffee	27.66		
<i>Polypedates</i> sp. juv.	R. F.	0.0		
	Cardamom	0.46		
	Coffee	0.62		

Bufo melanostictus. This is the only common toad encountered in the area. The toad has been observed to retreat in the crevices during the dry summer day. In 3 cases we also noticed more than four individuals using the common retreat sites, under a rock. Although the adults are rarely found near the water the juveniles were often encountered near the streams in coffee and cardamom estate streams. The relative abundance of the species in coffee plantation was estimated to be 1.09% were as in cardamom it was 0.3%. We did not sight any juveniles nor the adults toads along the forest streams.

Micrixalus saxicolus. This stream frog is mostly found adhering on to the surface of rocky outcrops within the streams. Black tadpoles of this species were observed to adhere firmly by oral suckers to the submerged rock surfaces within the fast flowing torrential streams. This frog was the most abundant species with 43.95% relative abundance in forest streams. On the other hand, it was completely absent from the streams of the coffee estate, and less abundant (23.38%) in the cardamom habitat (Fig. 1).

Fejervarya limnocharis. Commonly known as the cricket frog, this species was found to be more abundant in the coffee estate streams than in the other two habitats ($F_{2,39}=90.41$, $P=0.0001$). The species breeds during the early rain from June to August in the pools and streams that flow less rapidly.

Indirana beddomii. This is a litter frog that sometimes occurs along the streams. The frog has a habit of

urinating when disturbed and jumps to a considerable distance. Adults are found resting on the stem buttress of the larger trees in the cooler habitats. Although information on their habits is sparse, the species has been reported to be extremely common elsewhere (Daniel, 2002)

We observed the species to be more abundant along the forest streams (relative abundance of 18.8%), followed by the cardamom habitat (16.1%). Although the mean number of frogs did not vary significantly between the three habitats ($F_{2,39}=1.073$, $P=0.352$), the coffee habitat recorded the lowest abundance of the species (4.12%).

Euphlyctis cyanophlyctis. The skipper frog was found mostly along the coffee estate streams, but in relative low numbers (relative abundance= 0.55%). They were always sighted inside the pools of stream water. They were seldom found in swift flowing stream waters. They were never sighted in the forest streams and their number in cardamom estate streams was very low (Table 2).

Polypedates pseudocruciger. This is among the least studied species of the Western Ghats. Except for a distribution report (Daniels & Ravichandran, 1995), there is no other information on this species. We observed adults of this species along the streams in the rainy season. We recorded the calls of eight individuals from shrubs overhanging water bodies in two locations in the forest at 940 m elevation. We sighted 15 individuals

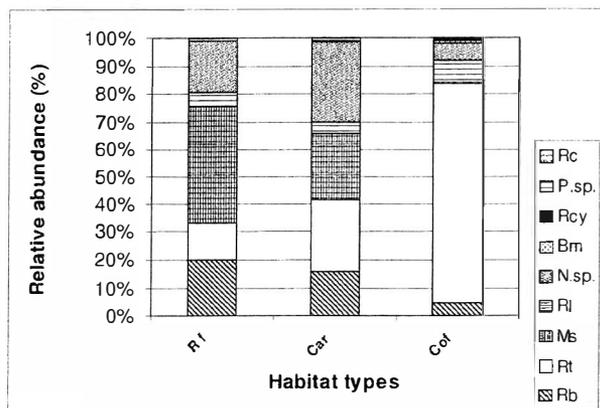


FIG 1. Relative abundance of frogs along the streams of Reserve forests (Rf), Cardamom estate (car), and Coffee estate (Cof) in the western slopes of evergreen forests of Western Ghats, Karnataka. Rc, *Rana curtipipes*; P. sp., *Polypedates pseudocruciger*; Rcy, *Euphlyctis cyanophlyctis*; Bm, *Bufo melanostictus*; N. sp., *Nyctibatrachus* sp.; Rl, *Fejervarya limnocharis*; Ms, *Micrixalus saxicolus*; Rt, *Rana temporalis*; Rb, *Indirana beddomii*.

along the stream in the cardamom estate and six individuals along the forest stream. We never sighted them along the stream transects of the coffee plantation.

We observed that anuran abundance in the natural forest and two human modified habitats varied significantly for most of the species (Table 2). *Rana temporalis* and *B. melanostictus* show significant variation in abundance between the three habitats, whereas *M. saxicolus*, *F. limnocharis* and *Euphlyctis cyanophlyctis* exist in almost equal numbers in the forest and cardamom streams. However, their abundance along the forest streams differed from that of coffee streams. The least affected species were *Nyctibatrachus*, *Indirana beddomii* and *Polypedates* (Table 2).

DISCUSSION

Rana temporalis, *Euphlyctis cyanophlyctis*, *Micrixalus saxicola* and *Nyctibatrachus* sp. are strictly aquatic. All the species listed in Table 2 breed in streams, and therefore the juveniles are found on stream banks. Hence, our observations of dry season sampling include the juvenile counts of *Bufo melanostictus*, *Polypedates* sp., and *Indirana beddomii*. However, juveniles remain along the stream for a brief period. Once the area starts receiving monsoon rains, they disperse into the forests, which are their feeding grounds, and therefore, adults are rarely found along the streams.

Our study shows that anuran species composition and relative abundance varied between the three habitats. The terrain and the general physical conditions of cardamom plantations were similar to that of the forest. The luxuriant growth and flowering of cardamom plantations is dependent on high rainfall, canopy cover for their soft tissues and a sloping terrain. The canopy cover and the litter cover are maintained intact for the retention of ground moisture in areas of cardamom

cultivation. On the other hand, the coffee plantations need more sunlight and therefore the canopy is cleared every year. The understory is removed and herbs are cleared in order to fertilize the coffee plants. The forests are modified completely in coffee cultivations and only a few trees are left intact. Due to the opening of the canopy, the annual mean temperature is high and the humidity is low in the coffee plantations when compared to the forest and cardamom plantations. Monthly average temperature ranges from 20.10° C to 24.03° C in the forest and the cardamom plantations, whereas the monthly average temperature in coffee plantations lies between 24° C and 28° C. The woody coffee plants are also susceptible to different insect stem borers. Therefore, these plantations are sprayed annually with insecticides such as Rogor, Nuvacron and Metasid (Daniels, 1991). Due to the sloping terrain and high rainfall, the residual insecticides may drain into the streams, which are used by anurans as breeding and retreat sites. Our results indicate that some species such as *Ansonia ornata*, *Micrixalus* sp. and *Polypedates* sp. are absent in such streams (Table 1). *Bufo melanostictus* was not encountered along the streams in cardamom and forest habitats. However, in the coffee estate, they were found to occur along the streams. This suggests that the climatic changes such as increased temperature and reduced humidity might result in a preference for cooler areas along the streams in the coffee estate. In concurrence with the observation of Dutta & Hejmadi (1981), we observed that *H. tigrina* was absent from all the three habitats studied (N=42 transects). It is possible that modified habitats such as coffee plantations, where opening of the canopy increases the temperature and reduces the atmospheric humidity, and with insecticide residues, might be detrimental for the existence of these species. Anurans like *R. temporalis* and *R. limnocharis* can be considered as hardy species, and are still more abundant in coffee estate streams than other two habitats.

The monoculture plantations, which have fragmented the forests and occupied the hilltops, except in the rainy season, do not support amphibian diversity (Krishnamurthy, 1999). Based on our data, we suggest that the monoculture plantations involving large-scale habitat modifications – as in case of coffee – affects anuran species composition and relative abundance in the rain forests of the Western Ghats. However, crops such as cardamom, when cultivated without modifying the natural habitat and grown as an intercrop in the forests of the Western Ghats, would form a suitable habitat for anurans. However, these natural forests in the area are under high human pressure. The present water crisis in the eastern plains has forced the state Government to propose projects for the diversion of water from the Western Ghats. Road constructions are exposing more forested and streams in the areas. This progressive habitat modification will convert the present forests into a drier zone and therefore many endemic species might go

extinct. Hence, it is vital to monitor anuran diversity and abundance in these modified forested areas. Further, the survival of anurans in the future could be assisted by managed forests in the form of cardamom estates.

ACKNOWLEDGEMENTS

We thank the Karnataka Forest Department and the estate owner Mr Umesh, for their support in conducting the field observations. We thank H. Venkatesh, T. O. Sajith, and Anil Kumar for their field assistance. We acknowledge the assistance of students of Bioscience Department, Hemangothri, Hassan, during the data collection. We are grateful to Mark Wilkinson, W. Wüster and two anonymous reviewers who provided useful comments on an earlier version of the manuscript. The financial support was provided by Declining Amphibian Populations Task Force, through the award of Seed Grant to S. N. K.

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HARMONIC DIRECTION FINDING: A NOVEL TOOL TO MONITOR THE DISPERSAL OF SMALL-SIZED ANURANS

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The suitability of harmonic direction finding for tracking of dispersing juvenile natterjack (*Bufo calamita*) and green toads (*B. viridis*) was evaluated in laboratory and field experiments. In a first step, dipole reflector tags were developed which combined low mass, small size and large detection range. The average mass was 114 mg, wire antenna length 42 mm and detection range usually varied between 2.5 m and 12.5 m – occasionally reaching 26 m – as assessed using a commercial portable scanning device RECCO 5000. In toads that had a snout-vent length of 22-24 mm, the mass of the reflector tag did not exceed 10% of the toad's body mass. Tags were externally attached by glueing to the dried dorsal skin of the toadlet. In a replicated laboratory experiment, almost all tags were shed 36 hr to 48hr after attachment. In 2001, 417 juveniles toads were equipped with reflector tags and their dispersal was studied in a natural habitat (Urmitz, Rhineland-Palatinate, Germany). The recovery rate of reflector tags was similar in *B. calamita* (35.9%, $n=33$) and in *B. viridis* (31.6%, $n=103$). The maximum distances between release and recovery site were 588 m in *B. calamita* and 665 m in *B. viridis*. Results obtained suggest that this new method is better suited for monitoring the migratory activity and habitat use of small terrestrial anurans than passive tagging systems presently in use, such as microtags and passive integrated transponders (PIT). Nevertheless, detection range is still too small to rival active monitoring systems such as radiotransmitters which remain unsuitable for small anurans.

Key words: *Bufo calamita*, *Bufo viridis*, postmetamorphic dispersal, passive tracking system

INTRODUCTION

Neighbouring populations are linked by dispersing individuals which do not only maintain gene flow but also counteract local extinction by recolonization of empty habitat patches (e.g. Hanski & Gilpin, 1997; Poethke *et al.*, 2003). Recent evidence even suggests that differences in dispersal ability among amphibians may play an important role in their sensitivity to the global decline phenomenon (Green, 2003). In many amphibian species, distances covered by dispersing juveniles by far exceed those of adults and thus, connectivity among populations mainly depends on the early terrestrial life stage (e.g. Dole, 1971; Breden, 1987; Sinsch 1991, 1997a). Nevertheless, our knowledge of the dispersal of juvenile amphibians is widely restricted to chance observations because of the absence of a suitable quantitative monitoring technique (e.g. Heyer *et al.*, 1994, Cooke *et al.*, 2004). Active systems such as radio tags are still too large and heavy for most species and the detection range of passive systems such as passive integrated transponders or microtags rarely exceeds a 0.2 m (e.g. Sinsch, 1997b; Ott & Scott, 1999). Consequently, a new technique closing the gap between the currently used passive and active tracking systems in amphibian population ecology is urgently needed.

Harmonic radar (Riley *et al.*, 1996) and harmonic direction finding (Mascanzoni & Wallin, 1986) are techniques to locate diode tags which convert the fre-

quency of an incoming radio signal to a harmonic frequency and reflect this harmonic as an outgoing signal. These techniques differ from active systems by the low mass and low price of tags and from passive ones by a larger detection range (Langkilde & Alford, 2002). Harmonic radar (radio detection and ranging) provides information on direction and distance of free-moving animals and has been successfully used to track bees (Riley *et al.*, 1996; Carreck *et al.*, 1999; Capaldi *et al.*, 2000), bumble bees (Osborne *et al.*, 1999) and moths (Riley *et al.*, 1998). However, radar studies require heavy equipment which is usually stationary. Harmonic direction finding (HDF) provides only information on direction, while the exact location of the tracked animal has to be assessed by homing-in with a portable detector. This method has been successfully applied to ground-moving carabid beetles (Hockmann *et al.*, 1989), snails (Janßen & Plachter, 1998) and snakes (Webb & Shine, 1997; Engelstoft *et al.*, 1999). Surprisingly, harmonic direction finding has not yet been used to monitor dispersing amphibians in which size limitations prevent the use of radio tags as in most insects. In a pilot study, we adapted this technique to track free-ranging juvenile *Bufo calamita* and *B. viridis* toadlets by developing suitable reflector tags which were small enough to minimize potential effects on behaviour and still provided an acceptable detection range (Leskovar & Sinsch, 2002). As results obtained on a low number of individuals were promising, the suitability of HDF tracking of toadlets was tested in quantitative field experiments. In this paper, we present (1) a detailed description of optimized reflector tags weighing about

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114 mg; (2) the corresponding detection ranges under laboratory and field conditions; (3) a suitable attachment method for short-term studies on small-sized toads; and (4) the recovery rates of tags and migratory distances quantified in field trials with 92 *B. calamita* and 325 *B. viridis* toadlets.

MATERIAL AND METHODS

Dispersal of juvenile natterjack (*Bufo calamita*) and green toads (*B. viridis*) was studied using a harmonic direction finding system. In 2001, juveniles (17.5–44 mm SVL) were collected in the vicinity of the breeding pond within a sandy gravel pit area near Urmitz (Rhineland-Palatinate, Germany; details on habitat structure in Sinsch *et al.* 1999). During daytime, juveniles mostly burrow into the sand or hide below stones and wooden boards. Migratory activity is mainly restricted to the first half of the night. Snout-vent length (to the nearest mm) and body mass (to the nearest 10 mg) of toadlets of each species were recorded prior to experimental treatments.

TRACKING METHODS AND EQUIPMENT

The HDF equipment used in this study consists of a commercial portable scanning device RECCO 5000 (size: 0.4 m × 0.1 m × 0.18 m, mass: 1.6 kg; supplier: RECCO AB, P.O. Box 4028, S-181 04 Lidingö, Sweden; price: ca. 5500 Euros) and self-built reflector tags (commercial silicium diode and antenna; price: ca. 0.50 Euro per unit). The hand-held RECCO 5000 includes a transmitter which emits a microwave (frequency: 917 MHz at 4–5 Watts), i.e. a directional signal, a detector for incoming microwaves which are transformed to an acoustic signal, and a headphone which allows an operator to evaluate amplitude and frequency of the acoustic signals. The self-built reflector tag used in this study is basically a dipole consisting of a diode connected with two antennas which reacts as the hollow antenna in commercial RECCO reflector tags (dipole design adapted from Janßen & Plachter, 1998). If the emitted microwave strikes the reflector diode, the incoming microwave is reflected with the doubled frequency (1834 MHz), detected by the receiver of the RECCO 5000 and transformed to an acoustic signal of correspondingly increased frequency (Mascanzoni & Wallin, 1986). The rechargeable batteries (NiMH, voltage: 6V) permit an operation period of about 3–4 hr.

Commercial reflectors provided by RECCO AB were too large and heavy to tag small animals. Therefore, in all tracking studies using HDF tags were specifically designed and assembled to meet size, shape and mass of the target organisms, some for implantation (Webb & Shine, 1997; Engelstoft *et al.* 1999), others for external attachment (Langkilde & Alford, 2002; this study). Here, we describe the design of the reflector tag developed for toadlets (Fig. 1). Tags consisted of a silicium diode (75V, 0.075A, device number 1N4148, size: 3.5 mm × 1.5 mm, mass: 44 mg), an isolated copper wire antenna and a copper foil antenna which were

soldered to either pole of the diode. The length of the wire antenna was 42 mm (diameter: 0.2 mm), the surface area of the foil antenna 15–20 mm × 2.5–3 mm, depending on the size of the toadlet which was tagged. The total mass of a reflector tag averaged 114 mg (range: 101–130 mg; Fig. 1). The optimum length of the wire antenna corresponds to $\lambda/4 = 42$ mm with wave length λ [cm] = speed of light c [300,000 km/h] / frequency of transmitter f [917 MHz]. The wire antenna was flexible, but did not kink. Wire and foil antenna were fixed in a T-like position to each other because the detection range decreased to about 50%, if the angle between the antennas was less than 90°. A copper wire which was wound round the diode five and a half times linked the poles. This coil provides a return path to prevent the rectifying action of the diode producing a DC charge distribution that would tend to bias the diode into the non-conducting state, but is of high enough inductance to avoid shorting out the 917 MHz signal (Riley & Smith, 2002). We obtained the maximum response, if neighbouring windings did not have direct contact. The optimal position of the wire coil was fixed using nail varnish. Combinations of different colours permitted batch marks indicating species and release date. Size and shape of the foil antenna were optimized empirically as well as wire length and the number of windings between the poles.

DETECTION RANGE AND ATTACHMENT PROCEDURE

The maximum distance at which reflector tags were detectable under field conditions was assessed for 10 randomly selected tags. Four combinations of tag location and vertical distance between tag and detector were tested: (1) tag placed on ground surface, detector waved at 1.5 m above ground; (2) tag buried 0.05 m below ground surface, detector at 1.5 m above ground; (3) tag placed on ground surface, detector at 4.5 m above ground; (4) tag buried 0.05 m below ground surface, detector at 4.5 m above ground. If the detection range was less than 4 m, the detector was moved towards the tag until receiving the first signal. To explore the potential effect of tag contact with toad skin on detection range, we also tested the combination: (5) tag attached to a toad and placed at the ground surface, detector at 1.5 m above ground.



FIG 1. Reflector tag consisting of a silicium diode, a copper foil antenna and a copper wire antenna. The coin has a diameter of 16mm.

External adherence of tags to the moist skin of amphibians using glue is a difficult task. Relying on the long-term experience of attaching magnets to the skin of European *Bufo* for orientation experiments, we chose cyanoacrylate glue (Sekunden Alleskleber, Uhu) which evidently does not have adverse effects besides an acceleration of moulting frequency (e.g. Sinsch, 1987, 1992). However, unpublished observations on other species suggest that cyanoacrylate glue may cause severe wounds in the skin of European *Rana* spp. and *Hyla arborea* and that *Bufo* skin is the exception to the rule. Reflector tags were attached to the dried dorsal skin of a toadlet by bending the flexible foil antenna around the toad's dorsum and fixing it with a small drop of glue (Fig. 2).

EXPERIMENT 1: ENDURANCE OF TAGGING UNDER LABORATORY CONDITIONS

Moulting is a limiting factor for the endurance of any tag attached to the skin of a toad. In *B. calamita* the period between two moults varies from 4.0 to 8.1 days (Sinsch *et al.*, 1992). As the endurance of tagging can hardly be estimated in the field, we chose a laboratory approach to quantify the time between attaching and shedding the reflector tags. A total number of 87 juvenile *B. calamita* and 189 juvenile *B. viridis* were collected during July and August in the study area. They were randomly assigned to four groups, fitted with reflector tags and kept at room temperature and a natural light-dark cycle in plastic boxes (0.41 m × 0.61 m × 0.22 m) with moist sand (0.04 m deep) until all had lost the tags. Individual numbers and species composition of treatment replicates was: (1) 50 *B. viridis* and 33 *B. calamita*; (2) 60 *Bv* and 25 *Bc*; (3) 39 *Bv* and 14 *Bc*; (4) 40 *Bv* and 15 *Bc*. The number of toads with attached tags per replicate was counted every 12 hrs. At the end of the experiment toads were kept another 4-5 days in captivity to look for potential adverse effects of the treatment. After this period they were released again in their natural habitat.

EXPERIMENT 2: DISPERSAL OF TAGGED JUVENILE *B. CALAMITA* AND *B. VIRIDIS* IN THEIR NATURAL HABITAT

Between 2 July and 21 October 2001, a total of 92 juvenile *B. calamita* and 325 juvenile *B. viridis* were

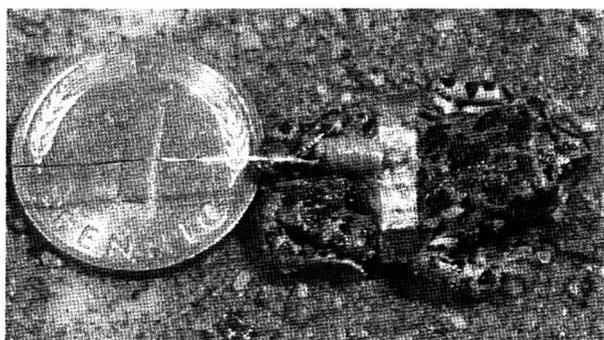


FIG 2. Juvenile *Bufo viridis* fitted with a reflector tag. The coin has a diameter of 16 mm.

collected during twelve 3-hr afternoon surveys in the study area. Collection and release date, group size and species composition were: 22 *B. calamita* and 26 *B. viridis* (2/07), 8 *Bc* and 18 *Bv* (10/07), 26 *Bv* (7/08), 24 *Bv* (17/08), 5 *Bc* and 40 *Bv* (24/08), 26 *Bv* (30/08), 5 *Bc* and 24 *Bv* (5/09), 2 *Bc* and 30 *Bv* (7/09), 24 *Bc* and 24 *Bv* (30/09), 5 *Bc* and 23 *Bv* (21/09), 5 *Bc* and 32 *Bv* (27/09), 16 *Bc* and 32 *Bv* (21/10). During and following the surveys toads were kept for 3-7 hr in plastic boxes (0.41 m × 0.61 m × 0.22 m) before release at sunset. In order to reduce handling stress the tags were attached in the field immediately before releasing the toadlets. As a group, the tagged individuals were placed on moist ground (sand) below a wooden board close to the breeding pond (Fig. 3). As the release site was the same for all experimental groups, the colour of the wire antenna indicated the species and the colour of nail varnish the date of release. Thus, we could identify any tag detected with respect to the corresponding species and the release group.

We did not intend to follow the individual paths of toads during their dispersal to avoid disturbance of the resting or migrating individuals and therefore, surveys to detect tags began 4-6 days after release, i.e. when almost all toadlets had already shed the tags. Surveys were exclusively performed during daytime and limited by the maximum battery charge to 6-7 hr (two batteries were available). The first search of tags following a release began at the release site with the receiver switched to maximum intensity and the detector antenna was held horizontally (Engelstoft *et al.*, 1999). In the close vicinity of the release site, the operator moved in a spiral by steadily increasing the distance to the release site by about 7-8 m per turn. However, the landscape did not permit an ideal spiral search at distances greater than about 120 m because large parts of the survey area were

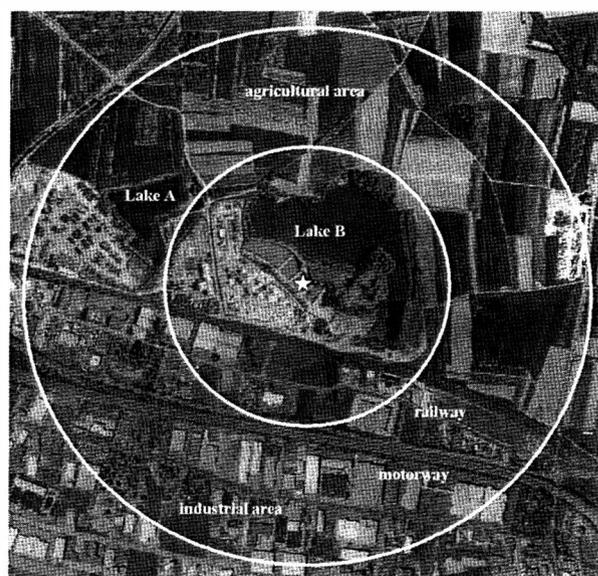


FIG 3. Aerial view on the study site. The star indicates the release site, the inner circle (diameter: 1 km) the most intensively surveyed area, the second circle (diameter: 2 km) the outer limit of the surveyed area.

covered by lakes, buildings, streets etc. (Fig. 3). Therefore, the accessible regions of the inner survey area (diameter: 1 km) were systematically searched in concentric circle segments until completely covered. A complete survey of the inner survey area lasted 18-20 hr. The survey within the outer search area (diameter: 2 km) was restricted to the area north of the railway because it soon became clear that toadlets did not cross the railway dam during the tagging period. As this area is considerably larger than the inner survey area, the outer region was scanned four times during 2001. A final complete survey to detect overlooked tags was performed two years after the last release, on 1 August 2003.

The most efficient method to detect the presence of reflector tags was waving the radar receiver at ca. 1.5 m above ground while moving through the habitat, because the signal intensity depends on the relative position of the linearly polarized receiver antenna to the tag (Janßen & Plachter, 1998). If the acoustic signal indicated the presence of a tag in the vicinity of the operator's position, its exact location was determined by a homing-in procedure, by progressively decreasing the sensitivity of the receiver until reaching the minimum. The location of buried tags often required time-consuming passing of soil through a sieve. Following tag identification its position was recorded as polar coordinates consisting of the direction to (Suunto compass) and distance from the release site (Bushnell laser range finder).

STATISTICAL ANALYSES

All data sets were tested for normal distributions by determining standardized skewness and kurtosis. Distributions of maximum detection distance per treatment and frequency of toads with attached tags did not differ from a normal distribution and were consequently compared using ANOVA and a multiple range test following Bonferroni correction or *t*-statistics. Means are always given with corresponding standard error. In contrast, distribution of dispersal distances was significantly skewed and compared using the Mann-Whitney *U*-test and the Kolmogorov-Smirnov test. We fitted a double-logarithmic regression model to size-mass relationships and calculated the Pearson product moment correlation. Significance level was set at $\alpha=0.05$. All calculations were performed using STATGRAPHICS Plus for Windows, version 5.0.

RESULTS

DETECTION RANGE

The location of the tag relative to the scanning device influenced the detection range significantly (ANOVA, $F_{4,45}=36.03$, $P<<0.0001$; Fig. 4). Individual features of a tag, e.g. size and shape of antennas also influenced detection range. Even if exposed to identical environmental conditions, the maximum detection distance of the best tag was 1.92 times larger than that of the worst performing one (e.g. 12.5 m versus 6.5 m). Maximum detection distance (\pm SE) averaged 9.45 ± 0.61

m, if tags were not attached to a toad and lay on the ground surface while the detector was moved at 1.5 m above ground. Any other test mode significantly reduced average detection range (Multiple range test, $P<0.05$; Fig. 4).

Nevertheless, maximum detection distance occasionally exceeded that of the standardized experiment. Preliminary tests following the assembly of tags within the University building yielded detection ranges of up to 30 m, e.g. if tags were placed on tables (75 cm above ground) and intervening structures between tag and detector were absent. In the field, maximum detection distance was 26 m in a reflector tag attached to a toad which hid within the moist sand of a slope, i.e. tag was at detector height. Nevertheless, the probability of detecting buried tags was lower than that of finding tags on the ground surface. Increasing the vertical distance between tag and detector did not increase detection range.

BIOMETRIC FEATURES OF TOADS

In experiment 1 snout-vent length of experimental juveniles ranged between 18.5-34.5 mm in *B. calamita* (median: 26.5 mm, $n=87$) and 19.5-31.0 mm in *B. viridis* (median: 23 mm, $n=189$); in experiment 2 the corresponding values were 22.0-44.0 mm in *B. calamita* (median: 32.5 mm, $n=92$) and 17.5-32.0 mm in *B. viridis* (median: 25 mm, $n=325$). The overall size-mass relationship significantly differed between the juveniles of *B. calamita* (regression model: $\log_{10}(\text{SVL, mm}) = -4.35 + 3.22 \times \log_{10}(\text{mass, g})$; $n=189$, $R^2=98.3\%$) and *B. viridis* (regression model: $\log_{10}(\text{SVL, mm}) = -4.53 + 3.32 \times \log_{10}(\text{mass, g})$; $n=514$, $R^2=98.6\%$) with respect to slope (ANOVA, $P=0.0072$) and intercept (ANOVA,

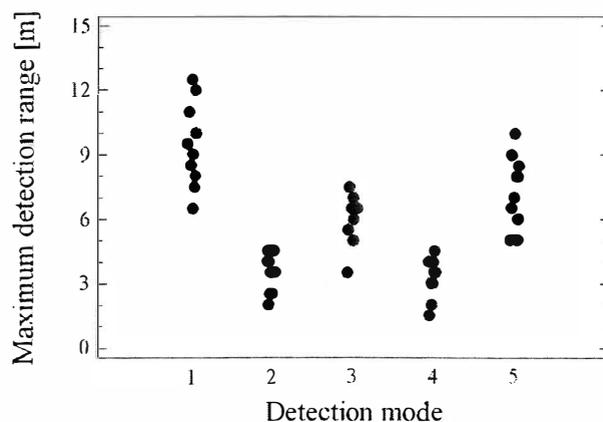


FIG 4. Maximum detection range of 10 reflector tags tested in five detection modes. (1) tag placed on ground surface, detector waved at 1.5 m above ground, detection range: 9.45 ± 0.61 m (mean \pm SE); (2) tag buried 0.05 m below ground surface, detector at 1.5 m above ground, detection range: 3.30 ± 0.31 m; (3) tag placed on ground surface, detector at 4.5 m above ground, detection range: 5.90 ± 0.37 m; (4) tag buried 0.05 m below ground surface, detector at 4.5 m above ground, detection range: 3.25 ± 0.29 m; (5) tag attached to a toad and placed at the ground surface, detector at 1.5 m above ground, detection range: 7.30 ± 0.53 m. If detection range was less than 4.5 m (modes 3 and 4), the detector was moved towards the tag until receiving the first signal. Each dot represents an individual tag.

$P < 0.0001$, Fig. 5). The tag load varied between 3.3% and 23.8% of individual body mass depending on toad size (Fig. 5). The snout-vent length at which the load amounted to ca. 10% of the individual body mass was 22–24.5 mm. The maximum individual tag load measured in experiment 1 was 19.4% in *B. calamita* (median: 7.0%) and 15.2% in *B. viridis* (median: 9.3%). The corresponding values for the field trials were 11.9% in *B. calamita* (median: 3.5%) and between 23.8% in *B. viridis* (median: 8.5%).

EXPERIMENT 1: ENDURANCE OF TAGGING UNDER LABORATORY CONDITIONS

The number of tags still attached to the skin significantly varied in time in both species (3-factor ANOVA; $F_{6,45} = 1079.94$, $P < 0.0001$; Fig. 6). In contrast, the time course of reflector loss did neither differ among the four replicate trials (3-factor ANOVA; $F_{3,45} = 1.02$, $P > 0.05$) nor between the two species (3-factor ANOVA; $F_{1,45} = 0.0$, $P > 0.05$). Single toads lost their tags during the first 12 hr following attachment. However, most toads shed the tags after 36 hr to 48 hr. Maximum duration of tag attachment was 72 hr. Attachment period of tags was unrelated to toadlet size. In a few individuals, skin which had been exposed to cyanoacrylate glue and/or the copper foil antenna was darker than normal. There was no mortality within a week after tagging.

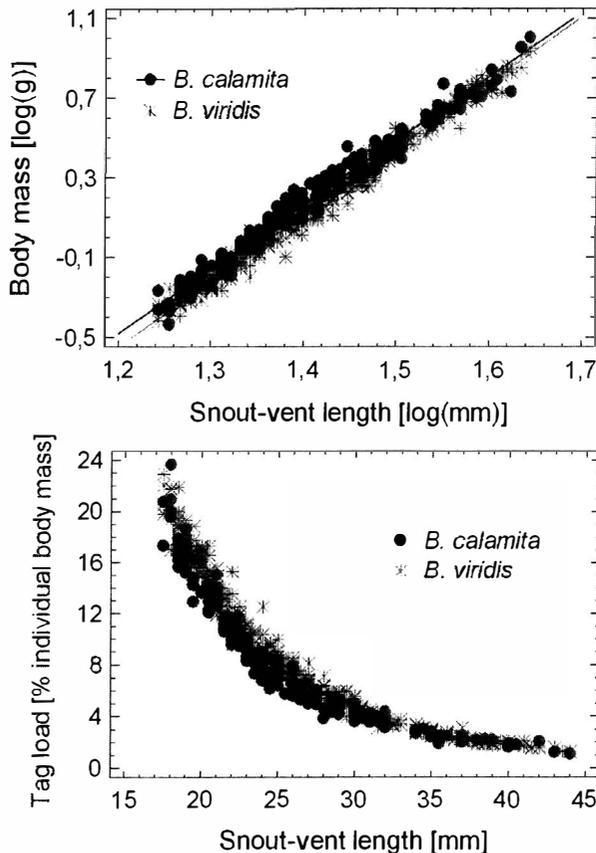


FIG. 5. Size-mass (top) and size-tag load (bottom) relationships in 179 juvenile *B. calamita* and 514 juvenile *B. viridis* studied in laboratory and field. For statistical details see text.

EXPERIMENT 2: DISPERSAL OF TAGGED JUVENILE *B. CALAMITA* AND *B. VIRIDIS* IN THEIR NATURAL HABITAT

With a few exceptions reflector tags were located after the toads had lost them. Average recovery rates per release did not vary significantly between *B. calamita* ($35.0 \pm 3.6\%$, $n=9$) and *B. viridis* ($31.0 \pm 3.0\%$, $n=12$; t -test, $t=0.86$, $P > 0.05$). The overall recovery rates of tags were 35.9% ($n=33$) in *B. calamita* and 31.6% ($n=103$) in *B. viridis*. Nine of the 136 recovered tags were detected during the final survey of the study area two years after the last release. In *B. calamita* five tags were found in cavities below stones which lay on the ground surface, six tags were buried up to 5 cm in sand, and 22 tags lay visible on the ground surface. In *B. viridis* one tag was located on a toadlet sitting in the pasture, five tags below stones, six buried in the sand, and 81 on the ground surface.

The maximum distance between release and recovery site was 588 m in *B. calamita* and 665 m in *B. viridis* (Fig. 7). Neither medians (68 m vs. 61 m; Mann-Whitney U -test, $U=1782$, $P > 0.05$) nor shape of the species-specific distributions differed among each other (Kolmogorov-Smirnov test; two-sided large sample K-S statistic = 0.769, $P > 0.05$). In both species, most of the

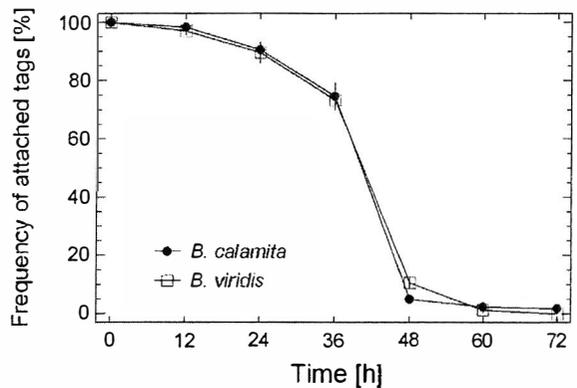


FIG. 6. Time course of reflector loss under laboratory conditions. Tags had been glued to the dorsal skin of 87 *B. calamita* and 189 *B. viridis*. Each symbol represents the average number (\pm SE, $n=4$ replicates) of tags still attached to a toad.

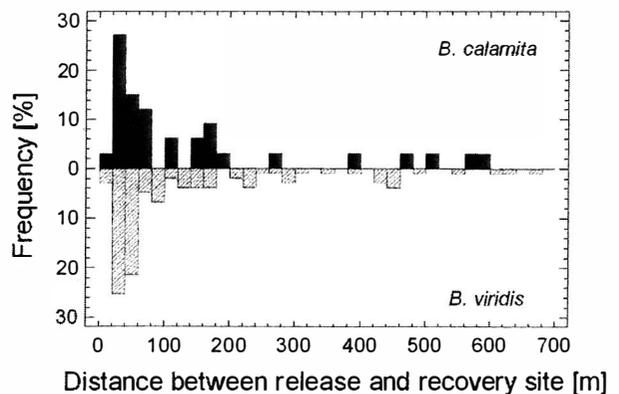


FIG. 7. Distances between release and recovery sites of attached reflectors (*B. calamita*: $n=33$; *B. viridis*: $n=103$). Data are presented as percent reflector tags within consecutive 20 m classes.

recovered reflectors (81.8% vs. 74.8%) were detected within a radius of 200 m around the release site. As the toadlets were not individually tagged, data on the potential effect of tag load on the dispersal distance were not available.

DISCUSSION

Harmonic direction finding has proved to be a suitable method to track non-climbing, free-ranging juvenile toads in their natural habitat, as demonstrated previously for carabid beetles (Hockmann *et al.*, 1989) and snakes (Webb & Shine 1997, Engelstoft *et al.* 1999). Compared to the few other available methods (e.g. microtags, passive integrated transponders), the small size and the low price of reflector tags combined with a detection range of about 7 m on average are intriguing advantages of HDF tracking (detailed discussion in Langkilde & Alford, 2002). Quantitative studies on the terrestrial dispersal of juvenile amphibians have come closer into the reach of population ecologists because now tagged toadlets can be located even if not visible to the observer. Before, it was necessary to locate the target organism by other means and to establish the presence of a tag in the already captured individual. Besides the obvious advantages of this method, there are several limitations which have to be considered, if planning a herpetological field study using HDF (Engelstoft *et al.*, 1999; Langkilde & Alford, 2002).

PREPARATION OF REFLECTOR TAGS

The tags which we describe here were optimized for HDF tracking of ground-moving juvenile toads and differ in size, mass and diode type from those used for other target organisms. When testing different types of diodes, we found that the detection range hardly depended on the diode type but mainly on antenna features. We used relatively large silicium diodes instead of Schottky diodes (e.g. Janßen & Plachter, 1998, Engelstoft *et al.* 1999) or germanium diodes (Langkilde & Alford, 2002) because they fitted best to our antenna design and smaller diodes would have required a more sophisticated electronic laboratory equipment. The dipole antenna design was chosen to facilitate attachment to the toad skin and to enhance signal reflection which is influenced by the alignment of tag and detector antennas (Janßen & Plachter, 1998). In contrast, studies on free-ranging snakes and captive hylids used a single antenna design with antenna lengths 70-130 mm (Webb & Shine, 1997, Engelstoft *et al.*, 1999, Langkilde & Alford, 2002). We refrained from using antenna of this length considering the small size of toadlets and the risk that the antenna becomes entangled with vegetation. As none of the recovered tags was caught within vegetation we assume that the external antenna length of 42 mm was an appropriate compromise between signal reflection capability and obstacle for movement within vegetation. Nevertheless, due to the external attachment

tagged toadlets are more conspicuous to visually hunting predators than untagged ones and may suffer from an increased predation risk. This applies to those individuals which do not burrow during daytime as occasionally observed in juvenile green toads.

Due to diode size and antenna design, the resulting tag mass of about 114 mg was greater than that reported for vertebrates for snakes (4 mg; Engelstoft *et al.*, 1999) or snails (69 mg; Janßen & Plachter, 1998). According to the recommendations for radio tracking, the total mass of a tag should not significantly exceed 10% of a toad's body mass (Richards *et al.*, 1994). Applying this rule-of-thumb to HDF tracking by far most of the juveniles were below this limit, but several individuals with 22-24 mm SVL and all smaller than 22 mm passed the limit, reaching tag loads of more than 20% body mass. We were unable to study the potential effects of tag load on locomotory activity as recovered tags could not be assigned to individuals, but it is probable that dispersal velocity will be negatively affected with increasing relative tag mass. Consequently, more light-weighted tags are needed to track metamorphs of *B. calamita* (6-11 mm SVL) and *B. viridis* (11-18 mm SVL) as well as small juveniles.

ATTACHMENT OF TAGS

Owing to the small size and the shape of toadlets a surgical implantation of tags into the peritoneal cavity as commonly practiced with radio transmitters but also in HDF studies on snakes was not considered (Stouffer *et al.*, 1983; Webb & Shine, 1997; Engelstoft *et al.*, 1999). External attachment of reflector tags to the moist skin of amphibians is a greater challenge than glueing a tag permanently to a chitin exoskeleton or to a snail-shell. We are aware of only two other studies on amphibians in which HDF tags were used to track adult *Litoria lesueuri* (Langkilde & Alford, 2002) and adult *Hyla arborea* (J. Pellet unpubl.). In these studies tags were fixed with an elastic waistband. The period in which frogs remain tagged is potentially longer using waistbands than using glued tags, however, waistbands irritate frogs causing them to move more often (Langkilde & Alford, 2002). Moreover, experiences derived from fixing mechanical tracking devices with waistbands suggest that they may cause skin lesions, if they are worn more than a week (e.g. Sinsch, 1988; Heyer *et al.*, 1994). We preferred tag attachment with cyanoacrylate glue because this glue does not seem to damage skin in the European toad species (*B. bufo*, *B. calamita*, *B. viridis*; Sinsch 1987, 1992). However, cyanoacrylate glue considerably reduced the period between two moults from 4.0-8.1 days to less than 2 days in *B. calamita* (Sinsch *et al.*, 1992; this study). A consequence of accelerated moulting is the temporal limitation of the tracking period to mostly two nightly activity periods, if the rate of glue-induced tag shedding estimated in the laboratory is similar to that in the field. However, considering that about 2/3 of the tags were not

recovered, the low endurance of the tag attachment guarantees that toadlets which are not recaptured will not suffer from potential long-term effects of the tag. The occasionally observed skin darkening was not limited to areas in direct contact with glue and more probably caused by the contact with the copper foil antenna.

DETECTION RANGE

The maximum distance at which HDF tags can be located using a RECCO detector is reported to depend on the antenna length and the tag's position over ground (Janßen & Plachter, 1998; Engelstoft *et al.*, 1999; Langkilde & Alford, 2002). Tags placed on the ground surface are detectable from ca. 3 m at 20 mm antenna length and from ca. 20 m at 180 mm antenna length. If the tag's position is about 0.6-0.8 m above ground, maximum detection distances of ca. 60 m are feasible. Signal amplitude is greatly attenuated, if HDF tags are buried and they may even become undetectable (Engelstoft *et al.*, 1999). Our data on ground-dispersing and burrowing toads corroborate these features into detail and suggest that under field conditions the average detection range is 7-8 m.

Further constraints influencing detection range are attenuation due to vegetation, to alignment of antenna and detector, and to the relative orientation of dipole antennas to each other (Janßen & Plachter, 1998; Engelstoft *et al.*, 1999; Langkilde & Alford, 2002). While vegetation is usually sparse in our study area and did not interfere, the optimal T-like orientation during laboratory trials is unlikely to be maintained in the field because the flexible wire antenna surely changes its relative position when a toadlet crosses vegetation or burrows.

These complex influences on the reflected signal's amplitude make tag location by harmonic direction finding a time consuming tracking method which requires a lot of manpower to collect quantitative data. As large detection ranges are rather the exception than the rule, the distance between parallel scanning paths should be 4-8 m to reduce the number of overlooked tags.

What can be learned from the field trial with free-ranging juvenile *B. calamita* and *B. viridis*? Reflector tags do not hinder juvenile toads to disperse more than 600 m within a maximum of four days, but most probably during two consecutive nights, i.e. HDF-tracking is well-suited to study short-time dispersal in the natural habitat. Available data on the velocity of dispersing juveniles of comparable size are scarce, but a maximum of 800 m per night in *Rana pipiens* (Dole, 1971) and 600 m within three weeks in *B. calamita* (Sinsch, 1997a) do not suggest that tags substantially modify dispersal behaviour. We do not know whether the velocity reported is representative for undisturbed toads or not because prior to experimental estimates toads were handled and displaced from their capture site. At least experimental displacement is known to increase distances covered

above the normal level during the first days following release (Sinsch, 1987). In adult *Litoria lesueuri* the presence of a tag and/or of a waistband was also observed to increase the number of movements and distance covered per hour (Langkilde & Alford, 2002). Thus, migratory velocity observed in this study – and probably in several others – may tend to overestimate that of undisturbed anurans.

The significance of a recovery rate of only about 1/3 of all tags in both species remains to be discussed. Several factors probably contributed to the failure to detect the missing 2/3 of tags: (1) some toadlets may have moved further than the documented 665 m. However, the number of wide-dispersers is not expected to be large because the recovery rate exponentially decreased with distance from the release site. (2) Some detectable tags have been probably overlooked during the surveys as in the natural habitat the scanning path may have deviated from the intended search pattern. As an additional nine tags have been detected two years following the final release, the proportion of overlooked tags may account for about 10-20% of the missing ones. (3) Other tags probably became undetectable due to either broken diode-antenna connection, unsuitable antenna position or simply for being lost in deep burrows. As these toads usually burrow during daytime, we assume that most of the missing tags are still within the inner study area but buried too deep to be detected with a RECCO device.

In conclusion, despite all of the shortcomings of HDF tracking of small anurans, we encourage population ecologists to use this method to obtain reliable field estimates on the direction and distance of postmetamorphic dispersal. It is surely an improvement on current methodologies – although the gap between the detection ranges of active and passive tracking systems has become smaller, it remains large.

ACKNOWLEDGEMENTS

The permissions for field studies and handling of the toads were issued by the Struktur- und Genehmigungsdirektion Nord, Koblenz. J. Wein provided the diodes and assisted in the technical improvement of the reflectors. B. Janßen, University of Marburg, shared with us his experience on harmonic direction finding. The helpful comments of J. Pellet and two anonymous reviewers on an earlier version of this paper are greatly acknowledged.

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SIZE-FECUNDITY RELATIONSHIPS AND REPRODUCTIVE INVESTMENT IN FEMALE FROGS IN THE PANTANAL, SOUTH-WESTERN BRAZIL

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The ovarian complement of anurans exhibiting different reproductive modes is highly diverse, and intraspecific variation in reproductive output of anurans is generally related to variation in female properties and/or environmental conditions. The size-fecundity relationships, reproductive investment, and correlation between ovary mass and fat body mass were investigated for females of an anuran assemblage in the Pantanal, Brazil. Female body size was positively correlated with clutch size among seven of the eight species analyzed. However, these results seem to be influenced by seasonal variation in fecundity. Interspecific size-fecundity relationships revealed that female SVL was positively correlated with clutch size and egg size regardless of reproductive mode. Among 11 species analyzed, the reproductive investment (RI: ovary mass relative to body mass) varied from 5.5 to 18%, and there were no differences among reproductive modes and activity patterns (explosive / prolonged). RI correlated negatively with female size. Among three *Leptodactylus* species examined, negative correlation between ovary mass and fat body mass was verified for two species. Variations in the reproductive strategies are closely related to the reproductive activity patterns and reproductive modes exhibited by individual species, but are also influenced by environmental conditions. However, regardless of reproductive mode or activity pattern, each species seems to exhibit a reproductive strategy that allows them to respond differently to the same environmental restrictions.

Key words: anurans, clutch size, fat body mass, reproductive investment

INTRODUCTION

As life history traits affect organism reproduction and survivorship, most of the interest in studying life history evolution comes from the different possible combinations of these traits which affect fitness (Stearns, 1992). Tropical anuran species exhibit a great diversity of reproductive patterns (Duellman & Trueb, 1986). The ovarian complement of anurans with different modes of reproduction is highly diverse (Salthe & Duellman, 1973). Generally, large species produce more eggs than smaller ones, and species that exhibit the generalized reproductive mode produce larger clutches than those with specialized modes. Furthermore, within a given reproductive mode there is a positive correlation between female body size and clutch size, and a negative correlation between clutch size and ovum size (Duellman, 1989). Information on size-fecundity relationships for anurans has been published by Salthe & Duellman (1973), Crump (1974), and more recently, by Lang (1995) and Perotti (1997).

Reproductive investment – also called reproductive effort – has been defined as the proportion of resources available to an organism of certain age, which is invested in reproduction during a defined period of time (Gadgil & Bossert, 1970). However, it is much easier to define than to measure such investment, and the most common

method used consists of establishing proportions as gonad mass relative to body mass or clutch volume relative to body volume (e.g. Crump, 1974; Lemckert & Shine, 1993; Perotti, 1997). In female frogs, which devote a great amount of energy into egg production, interspecific variation in reproductive investment is related to reproductive mode (Ryan, 1992). While intraspecific variation in reproductive output of anurans may be related to variation in female properties and environmental conditions (e.g. Berven, 1988; Lüddecke, 2002). However, comparative studies on reproductive investment and fecundity are scarce for Neotropical frogs inhabiting the seasonal Chaco-Pantanal domain (e.g. Perotti, 1997; Prado *et al.*, 2000).

The size-fecundity relationships and reproductive investment (RI) of female frogs from a Neotropical assemblage in the Pantanal, south-western Brazil, are herein described. As this region is markedly seasonal with a long dry period, main predictions tested in the present study were: (1) that RI will be greater in explosive breeders compared to prolonged breeders regardless of reproductive mode; and (2) that females of prolonged breeders will produce larger clutches in the wet season compared to the dry season. Regardless of reproductive mode, clutch size correlates negatively with egg size in anurans (e.g. Crump, 1974). Consequently, we expect little variation in RI among species exhibiting different reproductive modes. Furthermore, individual variation in ovary mass and fat body mass was also investigated for three *Leptodactylus* species.

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MATERIALS AND METHODS

STUDY SITE

The Pantanal is a sedimentary floodplain located in south-western Brazil, with an area of approximately 140,000 km² between the parallels 16°-22° S and 55°-58° W. The study was conducted in the municipality of Corumbá, MS, at the Pantanal Research Station, Federal University of Mato Grosso do Sul (19° 34'S - 57° 00'W). Climate is markedly seasonal ("Aw" type in Köppen's classification), with wet summers from October to April and dry winters from May to September. Rainfall concentrates from November to January, with an annual mean of 1215 mm at the study site, between 1995 and 1998. For the same period, mean annual temperature was 25.1 °C, varying from 15.8 °C (June 1996) to 29.5 °C (January 1998). The topography is plain, formed mostly by hydromorphic soils, causing drainage problems, that partially explains its tendency for periodic and prolonged floods (Por, 1995), which are common in the region from January to April. The region is characterized by open formations included in the Cerrado domain (savanna-like vegetation).

STUDIED FROG ASSEMBLAGE

The anuran assemblage at the study site is composed by 24 frog species belonging to four families: Bufonidae, Hylidae, Leptodactylidae, and Microhylidae, and the reproductive activity concentrates in the rainy season (November-January; Prado *et al.*, 2005). Herein we present data for 16 anuran species belonging to three families (Table 1), with three continuous breeders (*Hyla nana*, *Lysapsus limellus*, and *Leptodactylus*

podicipinus), seven prolonged breeders, reproducing for more than three consecutive months, and six explosive breeders. Five reproductive modes occur among the frog species, with the majority exhibiting the generalized aquatic mode, with eggs deposited in lentic water bodies and exotrophic aquatic tadpoles (Prado *et al.*, 2005; Table 1). Terrestrial modes are exhibited by *Phyllomedusa hypochondrialis*, which deposits eggs in leaves above water and tadpoles develop in lentic water, *Leptodactylus fuscus* and *Adenomera cf. diptyx*, which deposit eggs in foam nests in terrestrial subterranean chambers and tadpoles are carried to the pond subsequent to flooding.

FIELD WORK

Data were collected monthly between January 1996 and December 1998. Additional data were collected in the rainy season (October-March) in the years 1995, 2001 and 2002. Clutches were collected in the field whenever possible, but most clutches were obtained from amplexed pairs or estimated based on number and size of mature ovarian eggs from gravid females caught in the field.

Eggs of each clutch were counted (= clutch size) and individual egg diameter from different clutches was measured to the nearest 0.1 mm with an ocular micrometer in a Zeiss stereomicroscope. Snout-vent length (SVL) of individuals was measured with a caliper ruler to the nearest 0.1 mm. Body, mature ovaries, and ovarian fat bodies were measured on electronic balances to the nearest 0.001 g for the larger species, and 0.0001 g for the smaller ones, after being blotted to remove ex-

TABLE 1. Reproductive modes, reproductive activity patterns, and reproductive period for 16 anuran species in the south Pantanal (source Prado *et al.*, 2005). Reproductive modes: (1) eggs and exotrophic tadpoles in lentic water; (8) foam nest and exotrophic tadpoles in lentic water; (18) eggs on leaves above water; exotrophic tadpoles in lentic water; (21) foam nest in subterranean chamber; exotrophic tadpoles in lentic water (Duellman and Trueb, 1986); (3) eggs and early larval stages in foam nests in water-filled basins constructed by males; exotrophic tadpoles in lentic water (Prado *et al.*, 2002).

Family/species	Mode	Reproductive pattern	Reproductive period
HYLIDAE			
<i>Hyla nana</i>	1	continuous	Jan-Dec
<i>H. raniceps</i>	1	prolonged	Sep-Apr
<i>Lysapsus limellus</i>	1	continuous	Jan-Dec
<i>Phrynohyas venulosa</i>	1	explosive	Oct-Jan
<i>Phyllomedusa hypochondrialis</i>	18	prolonged	Sep-Mar
<i>Pseudis paradoxa</i>	1	prolonged	Oct-May
<i>Scinax acuminatus</i>	1	explosive	Oct-Mar
<i>S. fuscomarginatus</i>	1	prolonged	Jan-May
LEPTODACTYLIDAE			
<i>Adenomera cf. diptyx</i>	21	prolonged	Oct-Mar
<i>Leptodactylus chaquensis</i>	8	explosive	Oct-Mar
<i>L. fuscus</i>	21	prolonged	Sep-Mar
<i>L. podicipinus</i>	3	continuous	Jan-Dec
<i>Physalaemus albonotatus</i>	8	prolonged	Oct-Apr
<i>Pseudopaludicola cf. falcipes</i>	1	explosive	Nov-Mar
MICROHYLIDAE			
<i>Chiasmocleis mehelyi</i>	1	explosive	Nov
<i>Elachistocleis cf. bicolor</i>	1	explosive	Sep-Mar

cess liquid. Percentage of mature ovarian mass relative to body mass was used as a measure of the reproductive investment (RI).

DATA ANALYSIS

Variables were tested for normality (Shapiro - Wilk *W* test) before each analysis. Size-fecundity relationships were examined through linear regression analysis (Zar, 1999). Intraspecific relationships between clutch size and female SVL and clutch size and female body mass (body mass - ovary mass) were determined. Relationships between clutch size and female SVL, egg size and female SVL, ovary mass and female mass, and reproductive investment and female mass were compared

among different species. Considering allometric relationships between organ size and body size, to meet statistical assumptions, and to allow biological interpretation and comparison (King, 2000), we calculated linear regressions and correlations with log-transformed data. The reproductive investment (RI) was compared among different reproductive modes and between explosive and prolonged (including continuous) breeders. Comparisons of ovarian egg production in the dry and wet seasons were made for two continuous breeders, *Hyla nana* and *Leptodactylus podicipinus*. As most frog species disappear out of the reproductive season, correlation between ovary mass and fat body mass was examined only for three species, *Leptodactylus*

TABLE 2. Mean±SD female SVL and mass, clutch size (number of eggs per clutch), egg diameter, ovary mass, and reproductive investment (RI) for the anurans in the Pantanal, Brazil. Range and sample size in parenthesis. *Parameters based on ovarian eggs.

Family/Species	Female SVL (mm)	Female mass (g)	Clutch size	Egg diameter (mm)	Ovary mass (g)	RI (%ovary/body)
HYLIDAE						
<i>H. nana</i>	21.3±1.5 (18.8-23.8;13)	0.6±0.1 (0.3-0.7;13)	242±125 (120-551;15)	0.9±0.05 (0.8-1.0;20)	0.09±0.01 (0.07-0.10;5)	15.6±4.6 (10.7-21.4;5)
<i>H. raniceps</i>	60.2±4.4 (51.0-70.8;34)	12.3±3.4 (7.2-22.5;32)	1991±533 (1220-3096;23)*	1.3±0.10 (1.1-1.5;30)*	1.64±0.73 (0.94-3.04;6)	11.9±6.0 (7.4-23.8;6)
<i>L. limellus</i>	18.6±1.4 (16.0-23.0;45)	-	63±20 (27-117;18)	1.2±0.05 (1.1-1.2;16)	-	-
<i>P. venulosa</i>	77.4±6.0 (67.7-86.0;10)	34.5±10.8 (20.3-56.9;10)	3981±271 (3625-4310;6)*	1.7±0.11 (1.4-1.8;30)	3.9±1.22 (2.52-4.86;3)	8.2±1.3 (6.7-9.3;3)
<i>P. hypochondrialis</i>	43.0±1.5 (41.3-46.0;10)	4.0±0.5 (3.1-4.8;10)	89±30 (25-136;15)	2.1±0.12 (2.0-2.4;26)	0.66±0.28 (0.47-1.17;6)	16.2±5.6 (10.8-25.2;6)
<i>P. paradoxa</i>	57.5±8.0 (45.5-73.2;17)	27.2±13.5 (8.6-62.2;14)	1834±1,194 (1000-4624;8)*	1.4±0.08 (1.3-1.5;15)	1.82±1.40 (0.80-4.90;7)	5.5±3.1 (2.6-11.1;7)
<i>S. acuminatus</i>	43.1±2.0 (40.4-48.0;34)	6.5±1.2 (4.9-9.3;33)	879±274 (594-1352;9)	1.1±0.07 (1.0-1.2;65)	0.77±0.36 (0.27-1.70;21)	11.1±5.3 (3.9-26.4;21)
<i>S. fuscomarginatus</i>	22.0±0.9 (21.0-22.7;3)	0.5±0.0 (0.5-0.6;3)	337±132 (185-421;3)	0.9±0.05 (0.9-1.0;10)	-	-
LEPTODACTYLIDAE						
<i>A. cf. diptyx</i>	25.7 (1)	-	36 (1)*	1.4±0.14 (1.2-1.5;5)*	-	-
<i>L. chaquensis</i>	71.3±4.5 (60.7-80.1;50)	34.8±6.5 (21.9-49.0;50)	4936±1720 (3007-8375;32)*	1.5±0.09 (1.4-1.7; 5)	5.87±1.72 (2.97-8.92;26)	16.0±2.9 (9.9-21.2; 26)
<i>L. fuscus</i>	43.6±1.9 (40.0-46.2;21)	8.6±1.3 (6.9-11.3;21)	214±27 (185-248;4)	2.2±0.07 (2.1-2.3;12)	0.58±0.24 (0.18-0.87; 8)	6.8±3.3 (2.6-12.6; 8)
<i>L. podicipinus</i>	39.5 ± 2.1 (35.6-44.0;36)	6.1±1.0 (4.4-8.2;27)	210 2±442 (1750-2953;6)	1.1±0.05 (1.0-1.2; 20)	1.08±0.28 (0.66-1.34; 10)	15.0±2.6 (11.3-18.5; 10)
<i>P. albonotatus</i>	23.5±2.3 (20.1-27.0;41)	1.2±0.4 (0.5-2.3;37)	719 ± 433 (246-1562;19)	1.0±0.04 (0.9-1.0;43)	0.21±0.10 (0.12-0.52; 12)	16.6±4.3 (11.0-22.4;12)
<i>P. cf. falciipes</i>	14.3±0.8 (13.5-15.0;3)	0.30±0.07 (0.25-0.35;2)	32(1)	0.8 ± 0.02 (0.8-0.9;9)	-	-
MICROHYLIDAE						
<i>C. mehelyi</i>	23.8±0.6 (23.3-24.5;3)	-	217±33 (183-250;3)*	1.3±0,09 (1.2-1.4;30)*	-	-
<i>E. cf. bicolor</i>	26.1±1.5 (23.3-28.7;13)	1.4±0.4 (0.8-2.3;9)	478±279 (178-888;5)*	1.0±0.04 (0.9-1.1;15)*	0.32±0.18 (0.21-0.53;3)	18.0±4.5 (15.0-23.3;3)

chaquensis, *L. fuscus*, and *L. podicipinus*. These species occurred throughout the year and sufficient data were collected for them. When variables were not normally distributed, nonparametric tests were performed. Results were considered statistically significant at the level of $P < 0.05$.

RESULTS

SIZE-FECUNDITY RELATIONSHIPS

Data on clutch and egg size were obtained for 16 species (Table 2). However, due to the small sample size for some species, intraspecific relationships between clutch size (= number of eggs per clutch) and female SVL were only possible for eight species, and between clutch size and female body mass for six species (Table 3). Body size was positively correlated with clutch size in the majority of the species, with body mass explaining more variation in clutch size compared to SVL (Table 3). For *L. fuscus*, neither SVL nor body mass were significantly associated with clutch size. For *P. albonotatus* and *S. acuminatus* SVL was not associated with clutch size. For *L. podicipinus*, neither SVL nor body mass correlated with clutch size ($R^2=0.09$; $P=0.23$; $n=17$; and $R^2=0.12$; $P=0.21$; $n=15$; respectively). But when females were analyzed separately by seasons (dry/rainy), both SVL and body mass were highly correlated with clutch size in the rainy season (Table 3), period when peak of activity occurred for the species.

Interspecific size-fecundity relationships were also examined (Table 2). Clutch size was positively correlated with female SVL among the species compared ($R^2 = 0.60$; $P < 0.01$; $n=16$). However, the species with terrestrial reproductive modes, *P. hypochondrialis*, *A. cf.*

diptyx, and *L. fuscus*, exhibited much smaller clutches than expected (Fig. 1), and two species that deposit eggs in foam nests on the water, *L. podicipinus* and *P. albonotatus*, exhibited larger clutches compared to the remaining species. Egg diameter also correlated positively with female SVL ($R^2 = 0.43$; $P < 0.01$; $n = 16$). Species with terrestrial modes, mainly *P. hypochondrialis* and *L. fuscus*, exhibited larger egg sizes compared to the remaining species (Fig. 2). Excluding the three species with terrestrial modes, the relationship between body size and egg size became even stronger ($R^2=0.68$; $P < 0.01$; $n=13$).

REPRODUCTIVE INVESTMENT

Mean reproductive investment (RI), including all species and reproductive modes, was $12.8 \pm 4.4\%$ ($n=11$), ranging from 5.5 to 18% (Table 2). For the species exhibiting aquatic eggs, mean RI was $11.7 \pm 4.6\%$ (range=5.5-18%; $n=6$), for the frogs with aquatic foam nests, it was $15.9 \pm 0.8\%$ (range=15-16.6%; $n=3$), and for two species with terrestrial eggs, values of RI were 6.8% for *L. fuscus* and 16.2% for *P. hypochondrialis*. The reproductive investment was not significantly different among these three categories of reproductive modes (Kruskal-Wallis, $H=0.92$, $df=2$, $P=0.63$). For explosive breeders, mean RI was $13.3 \pm 4.5\%$ (range=8.2-18%; $n=4$) and for prolonged species it was $12.5 \pm 4.6\%$ (range=5.5-16.6%; $n=7$), showing no significant difference (Mann Whitney *U*-test: $W=12.0$; $P=0.70$). Ovary mass was strongly associated with female mass among the species analyzed ($R^2 = 0.91$; $P < 0.001$; $n=11$; Fig. 3). Results of linear regression analysis revealed a negative relationship between RI and female mass ($R^2=0.38$;

TABLE 3. Results of linear regression analysis between female SVL and clutch size and female body mass and clutch size for females of anurans in the Pantanal, Brazil. NS = non significant results.

Family/Species	log SVL (mm) vs. log clutch size	log body mass (g) vs. log clutch size
HYLIDAE		
<i>H. nana</i>	$R^2=0.59$; $P < 0.01$; $N=12$ $y = -4.0 + 4.8x$	—
<i>H. raniceps</i>	$R^2=0.25$; $P=0.03$; $N=23$ $y = -0.15 + 1.9x$	$R^2=0.34$; $P < 0.01$; $N=23$ $y = 2.6 + 0.57x$
<i>L. limellus</i>	$R^2=0.25$; $P=0.03$; $N=19$ $y = -2.8 + 3.5x$	—
<i>S. acuminatus</i>	$R^2=0.41$; $P=0.06$; $N=9$ NS	$R^2=0.51$; $P=0.03$; $N=9$ $y = 1.61 + 1.8x$
LEPTODACTYLIDAE		
<i>L. chaquensis</i>	$R^2=0.30$; $P < 0.01$; $N=26$ $y = -0.89 + 2.5x$	$R^2=0.57$; $P < 0.01$; $N=26$ $y = 2.0 + 1.1x$
<i>L. fuscus</i>	$R^2=0.19$; $P=0.21$; $N=10$ NS	$R^2=0.05$; $P=0.5$; $N=10$ NS
<i>L. podicipinus</i>	$R^2=0.65$; $P < 0.01$; $N=9$ $y = -4.0 + 4.6x$	$R^2 = 0.62$; $P=0.01$; $N=9$ $y = 2.2 + 1.5x$
<i>P. albonotatus</i>	$R^2=0.18$; $P=0.16$; $N=12$ NS	$R^2=0.42$; $P=0.02$; $N=12$ $y = 2.6 + 0.82x$

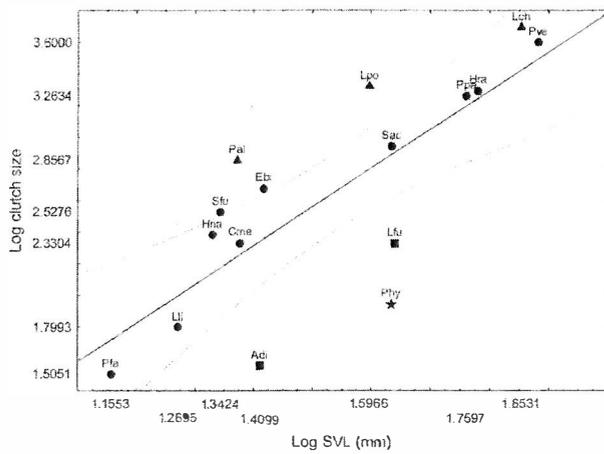


FIG. 1. Relationship between mean log female SVL and log clutch size for 16 anuran species in the Pantanal ($\log y = -1.1 + 2.4 \log x$). Species: (Adi) *Adenomera cf. diptyx*; (Cme) *Chiasmocleis mehelyi*; (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Lli) *Lysapsus limellus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Pfa) *Pseudopaludicola cf. falcipes*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*; (Sfu) *S. fuscomarginatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

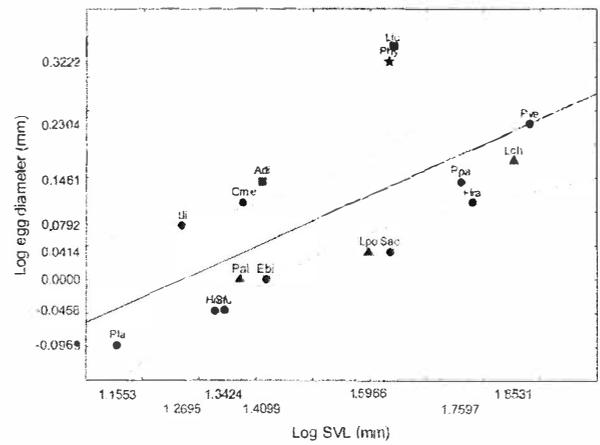


FIG. 2. Relationship between mean log female SVL and log egg size for 16 anuran species in the Pantanal ($\log y = -0.48 + 0.38 \log x$). Species: (Adi) *Adenomera cf. diptyx*; (Cme) *Chiasmocleis mehelyi*; (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Lli) *Lysapsus limellus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Pfa) *Pseudopaludicola cf. falcipes*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*; (Sfu) *S. fuscomarginatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

$P=0.04$; $n=11$; Fig. 4), i. e., females of larger species tend to invest less in ovaries. However, RI in females of *L. chaquensis*, an explosive breeder that deposits eggs in foam nests on the water, was much higher than expected (Fig. 4). In contrast, females of two species, *Pseudis paradoxa*, a prolonged breeder that exhibits the generalized aquatic mode, and *L. fuscus*, another prolonged breeder, but with a terrestrial reproductive mode, invested much less in ovaries (Fig. 4).

SEASONAL CHANGES IN FECUNDITY AND RELATIONSHIP BETWEEN OVARIAN MASS AND FAT BODY MASS

For two continuous breeders, *H. nana* and *L. podicipinus*, ovarian egg production in the wet and dry season was highly variable. Females of *H. nana* produced, on average, 250 ± 130 (range=139-551; $n=10$) mature ovarian eggs in the wet season and 218 ± 106 (range = 120-364; $n=7$) in the dry season, but this difference was not significant (Mann Whitney *U*-test:

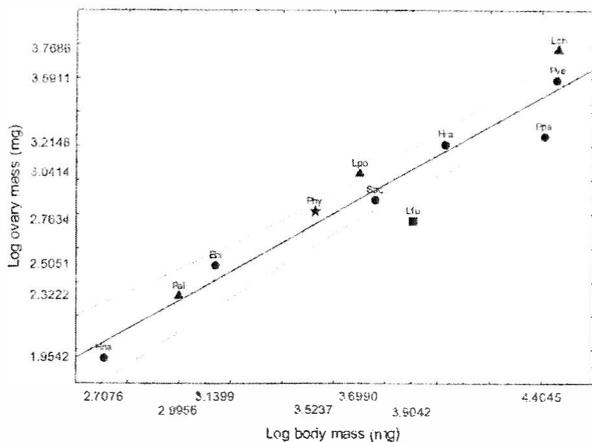


FIG. 3. Relationship between mean log female body mass and log ovary mass for 11 anuran species in the Pantanal ($\log y = -0.27 + 0.85 \log x$). Species: (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

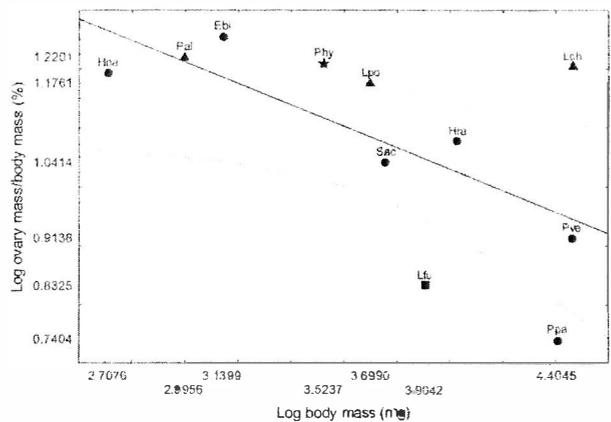


FIG. 4. Relationship between mean log female body mass and log ovary mass/body mass (reproductive investment) for 11 anuran species in the Pantanal ($\log y = 1.7 - 0.18 \log x$). Species: (Ebi) *Elachistocleis cf. bicolor*; (Hna) *Hyla nana*; (Hra) *H. raniceps*; (Lch) *Leptodactylus chaquensis*; (Lfu) *L. fuscus*; (Lpo) *L. podicipinus*; (Pve) *Phrynohyas venulosa*; (Phy) *Phyllomedusa hypochondrialis*; (Pal) *Physalaemus albonotatus*; (Ppa) *Pseudis paradoxa*; (Sac) *Scinax acuminatus*. Curved lines represent 95% confidence interval. Circles, eggs aquatic; triangles, aquatic foam nests; squares, terrestrial foam nests; stars, terrestrial eggs on leaves above water.

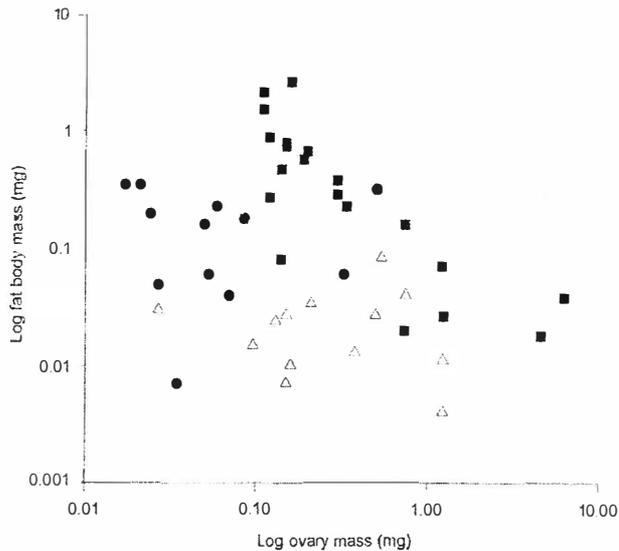


FIG. 5. Correlation between log ovary mass and fat body mass in females of *Leptodactylus chaquensis* (squares), *L. fuscus* (circles), and *L. podicipinus* (triangles) in the Pantanal, Brazil.

$W=28.0$; $P=0.49$). Females of *L. podicipinus* produced a mean of 2272 ± 464 (range = 1700 -2960; $n=9$) ovarian eggs in the wet season and a mean of 1766 ± 393 (range=1304-2526; $n=8$) in the dry season. For *L. podicipinus*, ovarian egg production in the wet and dry season differed significantly ($t=2.4$; $P=0.01$).

A significant negative relationship between ovary mass and fat body mass was verified for the explosive breeder *L. chaquensis* (Spearman rank correlation: $r_s = -0.74$; $P < 0.05$; $n=50$; Fig. 5) and for the prolonged breeder *L. fuscus* ($r_s = -0.69$; $P < 0.05$; $n=21$; Fig. 5). For the continuous breeder *L. podicipinus*, there was no significant correlation between ovary mass and fat body mass ($r_s = -0.16$; $P > 0.05$; $n=27$; Fig. 5).

DISCUSSION

SIZE-FECUNDITY RELATIONSHIPS

Although female size correlated positively with clutch size in most species (7/8; Table 3), body size explained between 25% (*H. raniceps* and *L. limellus*) and 65% (*L. podicipinus*) of the variation in clutch size. Female size is positively correlated with fecundity in many species of frogs (e.g. Berven 1988; Lemckert & Shine, 1993; Lüddecke, 2002), but egg production may exhibit considerable variation among females in a single population (e.g. Berven 1988; Lemckert & Shine, 1993). Besides size, other factors may influence female fecundity, as environmental conditions and female nutritional state (e.g. Ryser, 1988, 1989; Lemckert & Shine, 1993). In prolonged or continuous breeding species, egg production may also differ if measured at different times (e.g. early, mid, or late season) during the breeding season (e.g. Praderio & Robinson, 1990; Giaretta & Kokubum, 2004). Variation in the regression coefficients in the present study could be related to the fact that, for most species, samples included females and clutches collected in different years, or different seasons

(dry/rainy) in the cases of the prolonged and continuous breeders. In the present study, neither SVL nor body mass correlated with clutch size in *L. fuscus*, but in another population studied in northern Brazil (Martins, 1988) SVL and number of ovarian eggs were positively correlated. An explanation could be that for the population in the Pantanal, females collected in different reproductive seasons were analyzed together and in the study conducted in northern Brazil, only females from a single reproductive episode were analyzed. But such differences could also be due to multi clutching, or related to population differences, or even it is possible that more than one species are being currently identified under the name *L. fuscus* (Wynn & Heyer, 2001).

Our results on variation in fecundity of *L. podicipinus* and *H. nana* support seasonal predictions (described above). Females of *L. podicipinus*, a continuous breeder at the study site, produced less ovarian eggs in the dry season compared to the rainy season. Analyzing all available females and clutches of *L. podicipinus*, neither SVL nor body mass correlated significantly with clutch size. But when females were analyzed separately by seasons (dry/rainy), both SVL and body mass were highly correlated with clutch size. In contrast, the other continuous breeder, *H. nana*, did not exhibit differences in egg production between the dry and rainy season at the study site. When all females from all seasons were analyzed, body size was positively correlated with clutch size. Although both species are continuous breeders, our results suggest that they exhibit different responses to environmental changes. As for *L. podicipinus* in the present study, variation in egg production between dry and rainy seasons was also reported for another continuous breeder in the Pantanal, *L. limellus* (Prado & Uetanabaro, 2000). Such variation was also observed for *Colostethus trinitatus* in a seasonal region in Venezuela (Praderio & Robinson, 1990), and the authors suggested that prey availability may diminish during the dry season affecting female nutritional state. This explanation could be applied for *L. podicipinus*, or, as noted by Crump (1974), it is possible that females regulate egg production according to climatic conditions.

Considering the reproductive modes in amphibians, from the most generalized aquatic to the most terrestrial modes, number of eggs deposited tends to decrease while egg size increases (Salthe & Duellman, 1973; Crump, 1974; Duellman & Trueb, 1986). Such a negative correlation was verified in many studies on reproductive modes in anurans (e.g. Crump & Kaplan, 1979; Hödl, 1990; Perotti, 1997). Although only three terrestrial modes were analyzed herein, such tendency was also observed. The species *P. hypochondrialis*, which deposits eggs on leaves above water, and *Adenomera* cf. *diptyx* and *L. fuscus*, which deposit eggs in foam nests in subterranean chambers, displayed smaller clutches and larger eggs compared to species exhibiting aquatic modes (aquatic eggs and aquatic foam nests). Two species that exhibit aquatic foam

nesses, *L. podicipinus* and *P. albonotatus*, had relatively larger clutches compared to other species. Perotti (1997) compared the ovary size factor (Duellman & Crump, 1974), a method to measure fecundity which takes into account egg size, among species in different families and different reproductive modes in the Chaco, Argentina. Two leptodactylid species present in the Chaco – *L. chaquensis* and *Physalaemus biligonigerus* – that also deposit eggs in foam nests on the water, also had higher values of fecundity compared to other species (Perotti, 1997). It would be necessary to have data on other species with aquatic foam nests to test whether such larger clutches are phylogenetic determined or a common trait associated to this reproductive mode.

Interspecific analysis showed that female SVL was positively correlated with clutch size in the present study, as registered before for other frog assemblages (e.g. Crump, 1974; Lang, 1995). However, considering intra- and interspecific analysis, body mass and mature ovary mass showed a much stronger correlation. Regarding size-fecundity relationships, in comparison to SVL, body volume or mass give a more accurate measure of the size of the frog's internal cavity and more clearly defines the ovarian capacity of anurans (e.g. Crump, 1974; Lang, 1995; Prado *et al.*, 2000). Our results support previous studies cited above, with body mass explaining more variation in clutch size compared to SVL. Egg size and body size were not significantly correlated in a study conducted by Lang (1995). However, Lang (1995) tested the correlation with stream-breeding hylid frogs, which exhibited low egg size variation. All the stream-breeding species had relative large eggs, probably related to the water current of these habitats, which could explain the lack of correlation. When analysing species with different reproductive modes, depositing eggs at different habitat types, egg size correlates positively with body size (Perotti, 1997; present study). This fact may probably be related with many different selective pressures leading to differences in egg sizes.

REPRODUCTIVE INVESTMENT

Reproductive investment (RI), measured as percentage of ovary mass relative to body mass, varied from 5.5 to 18% among frog species in the present study. However, the RI did not differ significantly among the three categories of reproductive modes tested, neither between prolonged and explosive breeders. Percentage of clutch volume relative to body volume was examined for 23 species in different families and with different reproductive modes at Santa Cecilia, Ecuador (Crump, 1974). Similar to values observed for the frogs in the present study, relative clutch size at Santa Cecilia varied from 3.1 to 18.2%, the smallest value being that of the largest species, *Bufo marinus*, and largest percentage being that of the small treefrog *Hyla cruentomma*. Crump (1974) found an inverse relationship between the reproductive investment and body size, and so did

Perotti (1997), in Argentina. Such inverse relationship was also observed for the frog species in the present study. This negative trend indicates that as body size increases, proportionately less of the body size is accounted for by the ovary size, or less is invested in gonads. Crump (1974) suggested that probably this results from the fact that large species have a proportionately larger amount of supportive tissue.

The frog *L. chaquensis* invested more in gonads than expected, and *L. fuscus* and *P. paradoxa* much less. Ecological differences among these species mainly regarding reproductive activity patterns and number of clutches a female can deposit during a single reproductive period may explain such differences. Many studies showed that individual females can produce more than one clutch per reproductive season (e.g. Perrill, 1983; Lemckert & Shine, 1993). Based on presence of immature ovarian eggs in nearly every gravid female, Crump (1974) suggested that females breed repeatedly throughout the year in the Neotropics. Such situation was also observed for all the species in the present study (C. P. A. Prado, pers. obs.), and it is also suggested that females of all studied species may produce more than a clutch per season. But the number of clutches a female can produce may be under the control of environmental conditions as well as the species reproductive activity pattern. In contrast to other explosive breeders in the Pantanal that breed many times during the rainy season (Prado *et al.*, 2005), *L. chaquensis* is an explosive breeder that only breeds during the first heavy rains in the rainy season (Prado *et al.*, 2000), a condition that is rare at the study site. As a consequence, females of this species may not have the opportunity to lay many clutches during a single breeding season, which could lead to a high reproductive investment. In contrast, *L. fuscus* and *P. paradoxa* are prolonged breeders that reproduce continuously for more than six consecutive months at the study site. Females of these species may lay many small clutches during a single season, which would explain the extremely low investment in gonads.

SEASONAL CHANGES IN FAT BODY MASS IN LEPTODACTYLUS

In amphibians and reptiles fat is stored in special organs, the fat bodies, located anterior to the gonads, and their sizes are good indicators of the nutritional state of the organism (Jorgensen, 1992). Three *Leptodactylus* species exhibited differences regarding patterns of fat deposition in the present study, indirectly indicated by the correlation between fat body mass and ovary mass. For *L. chaquensis* and *L. fuscus*, fat body mass correlated negatively with ovary mass, i.e. females with ovaries containing eggs exhibited smaller fat bodies. However, for *L. podicipinus*, fat body mass was not correlated with ovary mass. In temperate regions, fat bodies in anurans are primarily known to serve as nutritional reserves, mainly during hibernation (Saidapur & Hoque, 1996). However, involvement of fat bodies in game-

togenesis has been reported for many species both in temperate (e.g. Long, 1987) and in tropical regions (e.g. Pancharatna & Saidapur, 1985; Saidapur & Hoque, 1996). An inverse relationship between fat body size and ovary size was reported for the temperate frog *Acris crepitans* (Long, 1987) and for the tropical frog *Rana cyanophlyctis* (Pancharatna & Saidapur, 1985), suggesting that lipids are used for vitellogenesis, as for *L. chaquensis* and *L. fuscus* in the present study. For *L. podicipinus*, fat body mass did not correlate negatively with ovary mass. In a study with *Bufo woodhousei* (Long, 1987), it was observed that although fat body size did not correlate negatively to ovary mass, lipid reserves were mobilized for egg production, and such a situation could be the case for *L. podicipinus* in the present study. Differences in fat deposition patterns among the *Leptodactylus* species may be due to ecological differences in reproduction. While *L. chaquensis* – an explosive breeder – and *L. fuscus* – a prolonged breeder – only reproduce in the rainy season, *L. podicipinus* reproduces throughout the year, which could explain the lower variation in lipid reserves throughout the year.

The Pantanal is included in the Cerrado-Caatinga-Chaco domain (Duellman, 1999), which is characterized by a subhumid to semiarid climate, high temperatures, and low rainfall, that falls mainly in a short rainy season. The climate is markedly seasonal in the Pantanal, with a defined wet period with unpredictable rains and a long dry season (Por, 1995). Floods are common in the region, but the exact period and intensity of floods may vary from year to year. Results herein described for the frog assemblage in the Pantanal suggest that variations in reproductive traits – as variation in egg production, pattern of fat deposition, and reproductive investment – although influenced by reproductive activity patterns and modes of reproduction, seem to be strongly influenced by environmental conditions. However, the most important conclusion is that, regardless of reproductive mode or activity pattern, each species seems to exhibit a unique reproductive strategy which allows them to respond differently to the same environmental restrictions.

ACKNOWLEDGMENTS

We thank J. P. Pombal Jr. and M. Martins for the valuable suggestions on the manuscript, E. A. Pedroso, E. Pereira, E. Q. Gomes, M. Uetanabaro, and P. Landgraf Filho for field assistance, and L. F. Toledo for the help with the figures. This study was made possible by the logistical support of Base de Estudos do Pantanal Universidade Federal de Mato Grosso do Sul. C. P. A. Prado acknowledges CNPq for financial support (proc. 521746/97 3/NV) and graduate fellowships (procs. 351228/97 7, 140397/2000 0), and FAPESP for post-doc fellowship (proc. 04/00709-0). C. F. B. Haddad acknowledges CNPq and Biota FAPESP (proc. 01/13341-3) for financial support conceded to the Laboratory of Herpetology, Department of Zoology, I. B., UNESP, Rio Claro, SP, Brazil.

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Accepted: 12.10.04

EMBRYONIC EXTERNAL NARES IN THE MICROHYLID *ELACHISTOCLEIS OVALIS*, WITH A REVIEW OF NARIAL DEVELOPMENT IN MICROHYLID TADPOLES

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Microhylids have the nares closed during most of tadpole life. In the microhylid *Elachistocleis ovalis* the external nares initially form in late embryonic stages but the nares then close when independent feeding begins and re-open at the start of metamorphosis. Examination of other microhylid species suggests that this may be a general feature. Given the uses of the olfactory system in tadpoles (e.g. kin recognition, predator detection), closure of the system in microhylids is a curious feature which needs further investigation.

Key words: anuran, development, nostrils, olfaction, Microhylidae

INTRODUCTION

Paired ectodermal placodes that form near the tip of the snout of anuran embryos at Gosner (1960) stage 17 are the earliest signs of the olfactory organs. The placodes invaginate to form olfactory pits (presumptive external nares), and soon after hatching each pit begins to divide into the anteromedial vomeronasal organ and the posterolateral principal cavity. The lumen of the principal cavity extends towards the roof of the buccal cavity and breaks through to form the choana (internal naris) by Gosner stage 28 in *Xenopus laevis* (Hansen *et al.* 1998; staged using the approximation table in McDiarmid & Altig, 1999). Klein & Graziadei (1983) showed that the olfactory placode is composed of a superficial cell layer which forms the supporting cells of the olfactory epithelium, with microvillate apical surfaces, and a deep layer that becomes sensory cells with ciliated apical surfaces.

It has been known for some time that the olfactory development of larval microhylids is unusual. Parker (1934) noted that the external nares of free-living microhylid tadpoles "do not appear until shortly before metamorphosis." Wassersug (1980) examined the internal oral anatomy of three species of microhylid tadpole (stages 32-37) and noted that the internal narial depressions were not perforated in any case. Haas (2003) concurred with this conclusion from examination of six more microhylid species, noting that the nostril passages became open only during metamorphosis, and that closed nostrils are one of a set of derived characters that distinguish microhylid tadpoles from those of other groups.

Altig & McDiarmid (1999) briefly reported that embryos of the tadpoles of *Gastrophryne carolinensis* and *Microhyla heymonsi* had external narial openings; they found that the external openings closed at later stages, and remained closed during most of larval development.

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During a study of the external features of embryos and larvae from a wide range of anuran taxa, we made similar observations in the microhylid *Elachistocleis ovalis* (Schneider). Here we present our observations on this species and re-examine the specimens reported by Parker (1934).

MATERIAL AND METHODS.

Eggs and tadpoles of *E. ovalis* were collected from temporary pools in Trinidad over a number of years. Embryos were incubated in well-aerated dechlorinated tapwater at 28°C in the laboratory and fixed in 2.5% glutaraldehyde in phosphate buffer. Tadpoles were grown in particle-rich pond water and fixed in Bouin's fluid at stages before and around metamorphosis (Gosner 37-44). Specimens were prepared by conventional methods for both scanning electron microscopy (SEM) and light microscopy using semi-thin resin embedded sections stained with Toluidine Blue or wax sections stained with PAS. For comparison, larvae of two species with primitive olfactory organ development (*Bufo bufo*, collected in the UK; *Hyla crepitans*, collected in Trinidad) were also fixed for light microscopy. Microhylid embryos and larvae held in the collections of the Natural History Museum, London (BMNH) were examined with a dissecting microscope.

RESULTS

As viewed by SEM, the positions of the nasal placodes of *E. ovalis* were first detectable as flattened areas dorsal to the oral invagination at stage 18 but became more obvious as invaginations at stage 19 when the embryos hatched. By stage 20, each external naris was a circular pit with a flattened base composed of densely packed cells, most of which bore short apical microvilli, but some of which were ciliated. The pits were deeper by stages 21-22 and remained distinct through stages 23-24, but by stage 25, they had disappeared; their previous positions were faintly visible as a circular arrangement of the cells (Fig. 1a-e). Although

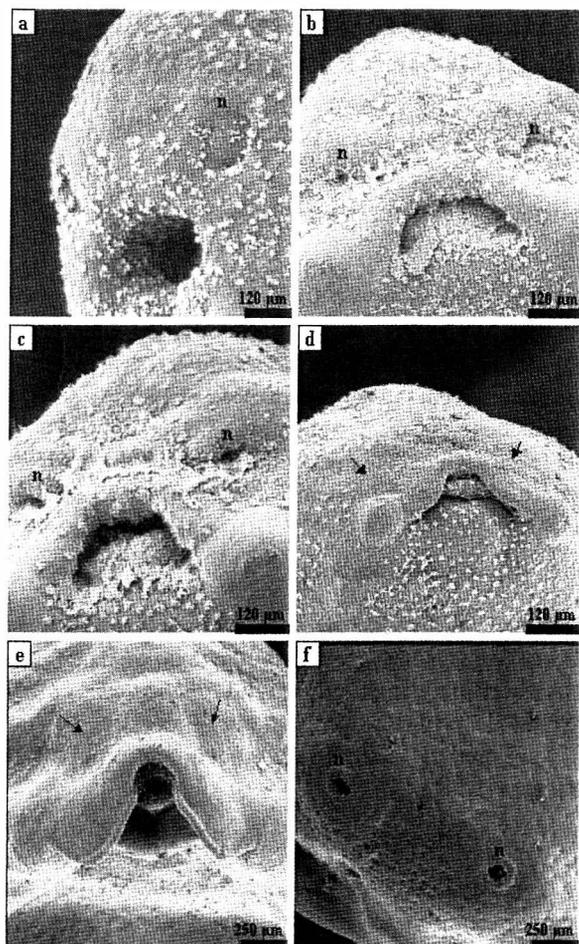


FIG. 1. Scanning electron micrographs of *E. ovalis* at embryonic and early metamorphic stages showing external nares. a: stage 20, b: stage 23, c: stage 24; d: stage 25; e: stage 25-26; f: stage 42. Labels: n - external nares; arrow - positions of former external nares. Structures below the nares in a-e are the central mouth and lateral adhesive glands.

they had a few ciliated cells in their proximity, these late embryonic/early larval external nares were not densely surrounded by ciliated cells, as Nokhbatolfighahai & Downie (in press) report for the external nares of many other tadpole species. At stage 37, there were no signs of external nares. At stages 38/39, a pair of surface elevations was visible at the expected positions of the external nares but openings were absent. By stage 42, the external nares were clearly open (Fig. 1f).

In *Bufo bufo* and *Hyla crepitans* (Fig. 2 a-c), the external narial openings connect via an olfactory canal with a thickened olfactory epithelium to the internal nares in stage 26 tadpoles. In *E. ovalis*, the olfactory pits at stage 23 have a basal thickened olfactory epithelium; by stages 25 and 27, the external opening has closed but there is an internal opening and an elaborately thickened olfactory epithelium.

Table 1 shows data from specimens in the BMNH together with our data from *E. ovalis*. Early larval stages were available only for *Kalophrynus pleurostigma*, and these confirmed the presence of nasal pits at stages 19-

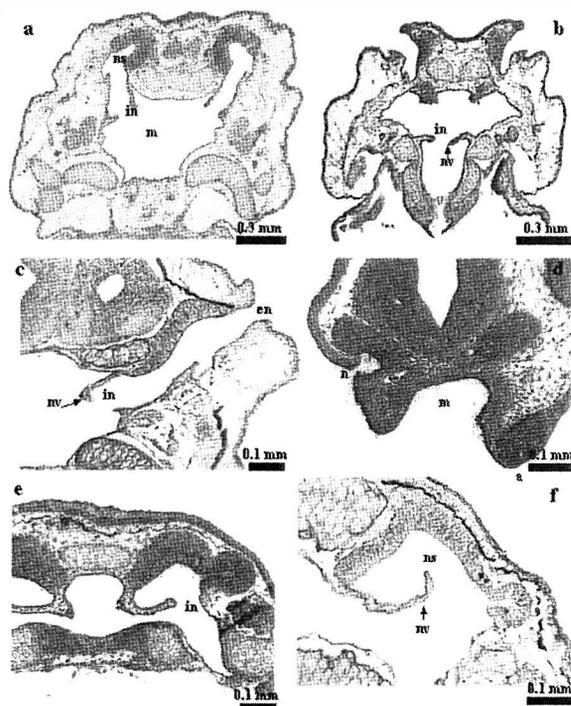


FIG. 2. Sections through the nostril region of three species of early larvae. a, b: *B. bufo*, stage 26, two sections through the same tadpole to show internal nares (a) and external nares (b). c: *H. crepitans*, stage 26, section showing both internal and external nares. d: *E. ovalis*, stage 23 showing external naris with thick olfactory placode at its base. e: *E. ovalis*, stage 25 showing internal naris and olfactory epithelium: external naris now closed. f: *E. ovalis*, stage 27 showing further development of the olfactory epithelium. Labels: a - adhesive gland; en - external naris; in - internal naris; m - mouth cavity; n - nostril; ns - nasal sac; nv - nasal valve. All figures photographed from PAS-stained wax sections, except d, semi-thin Toluidine Blue-stained resin section.

25, and their later absence. Absence of nostrils as early as stage 25 was confirmed for three species. The precise stage at which nostrils opened in later tadpoles varied from stage 39-41, based on a few available species.

DISCUSSION

In *E. ovalis*, the nasal pits develop at the same stage as in other anurans, but they close externally around the start of independent feeding and re-open at the start of metamorphosis. Internally, *E. ovalis* has an olfactory epithelium similar to that in other anurans, at least in early tadpole stages, and open internal nares; we did not examine the internal nares after stage 27.

Parker (1934) wrote that the "external nares do not appear until shortly before metamorphosis" although he examined the late embryonic stages only of *Kalophrynus pleurostigma* and *Microhyla borneensis*; in the former, he noted shallow nasal pits in recently hatched larvae, which we confirmed as stages 19-25. He made no mention of nasal pits in *M. borneensis* hatchlings, and these specimens are no longer available.

TABLE 1. Nostril appearance in developmental stages of different species of Microhylidae.

Species	Stages nostrils visible	Stages nostrils not visible	Stages nostrils not clear
<i>Calluella guttulata</i>	44, 46	37, 38, 42	43
<i>Glyphoglossus molossus</i>	42, 44	25, 26, 30, 35, 36, 37, 37/38	40, 41
<i>Kaloula pulchra</i>	39, 42/43, 45	38	38/39
<i>Kalophrynus pleurostigma</i>	19/20, 24, 25	29/30	-
<i>Chaperina fusca</i>	42	29, 32	25, 26
<i>Microhyla berdmorei</i>	41, 42, 42/43, 44	25, 34, 35, 37, 37/38	39/40, 40
<i>Microhyla butleri</i>	-	36, 36/37, 38	40
<i>Microhyla heymonsi</i>	-	36, 36/37, 37, 38	-
<i>Microhyla achatina</i>	-	37, 39	-
<i>Microhyla pulchra</i>	40, 43, 46	34	-
<i>Microhyla ornata</i>	-	25, 36, 37, 38, 42	43/44
<i>Microhyla rubra</i>	43, 44	37	-
<i>Elachistocleis ovalis</i>	19-24, 42	25, 26, 27, 37, 38/39	-

Altig & McDiarmid (1999; personal communication) reported open nares in embryos of *Gastrophryne carolinensis* and *Microhyla heymonsi* with later closure that persisted through most larval stages. They also stated that the external nares of nidicolous species of the sub-family Cophylinae are open at least at the surface throughout development, but it is not known if this applies to other nidicolous microhylids. Blommers-Schlösser (1975) reported that nostrils appeared late in the development of the swimming tadpoles of the cophyline *Platyhyla grandis*, though still before metamorphosis.

Open external nares have been reported from swimming tadpole stages in a few microhylid species. Berry (1972) showed open external nares from stage 26 onwards in *Phrynella pollicaris*; Mohanty-Hejmadi *et al.* (1979) described nostrils, without comment, in all stages of *Uperodon systoma* from late embryos onwards. Kirtisinghe (1958) described nostrils in tadpoles of *Ramanella palmata* and *Kaloula pulchra taprobanica*, but did not give stages. Parker (1934) observed late stage *Ramanella* tadpoles, but found nostrils opened only in *K. pulchra* tadpoles with well-developed hindlimbs (later than stage 38; our Table 1). Wassersug (1980) examined the internal narial depressions of three species of microhylid tadpoles from stages 32-37 and found that they were not open in any case.

It would clearly be helpful to examine embryonic and early larval stages in more microhylid species but, if it is possible to generalise from the present data, external nares develop normally in microhylid embryos. These apertures then close about the time of independent feeding in nearly all species with free-swimming tadpoles but remain open in species with larger eggs developing on land. Internal nares also form normally, then close in at least some tadpole species and re-open at metamorphosis.

Why does the olfactory system open up in the first place, and why does it later close off? It is likely that the formation of the nasal placodes and their subsequent invagination to form nasal pits is an essential early stage in

the chain of developmental interactions that forms the olfactory organs. Therefore, even if the olfactory system is to remain unused during most of tadpole life, it has to undergo these initial stages. Wassersug (1980) suggested that the closure of the internal nares in microhylid tadpoles was related to the efficiency of their buccal pumps; a reduction in the number of openings removes sources of leaks. This may be so, but it is surprising that this is a big enough advantage to overcome the loss of a sensory modality and it has yet to be established in how many microhylid species internal narial closure occurs. The sensitivity and uses of the olfactory system in tadpoles has not been fully investigated (review by Dawley, 1998) but it is responsible for chemical mediated kin recognition in some species and predator detection in others. *E. ovalis* tadpoles do not form shoals (Downie, personal observations), so it is unlikely that they show kin recognition, but the ability to detect predators would seem of importance. The stages at which the anuran olfactory system becomes functional has not been fully established, but in *X. laevis*, there is evidence for functionality just before the start of independent feeding (Hansen *et al.*, 1998). Assheton (1896) reported a powerful cilia-generated current sweeping into the olfactory pits of recently-hatched *Rana temporaria* larvae. If the olfactory cells are functional at this stage, the current may bring useful sensory information which could then be available to microhylid larvae in the early post-hatching stages when their external nares are still open.

ACKNOWLEDGEMENTS

We thank Margaret Mullin and Ian Montgomery for technical assistance and advice, the late Professor Peter Bacon and colleagues at the University of the West Indies for laboratory space, Barry Clarke and Mark Wilkinson for their help in accessing and studying specimens at BMNH and Ronn Altig for sharing unpublished results and commenting on a draft of this paper. We are also grateful for a comment by Alex Haas on the development of the internal nares in microhylids. JRD acknowledges financial assistance from the Carnegie

Trust and the University of Glasgow; MN carried out this work while on a Government of the Islamic Republic of Iran postgraduate scholarship.

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Accepted: 18.10.04

REPRODUCTIVE BIOLOGY OF *ATRACTUS RETICULATUS* (BOULENGER, 1885) (SERPENTES, COLUBRIDAE) IN SOUTHERN BRAZIL

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The reproductive biology of *Atractus reticulatus* was studied based on the analyses of 135 specimens from preserved in collections, and on field observations in Rio Grande do Sul State, southern Brazil. The snout-vent length (SVL) of mature females was significantly larger than the SVL of mature males, and the tail length (TL) of mature males was significantly longer than the TL of mature females. Analysis of neonate body size, hatching time and seasonal distribution of body size classes (SVL) suggests that males reach sexual maturity at eight to 10 months old (SVL c. 198 mm) and females at 11 to 12 months old (SVL c. 242 mm). The reproductive period begins in late August, with clutches laid in November, December and January, and hatching occurring from January to March. Fecundity is low: the number of vitellogenic follicles varied from 2 to 6, and the number of eggs from 1 to 3. Relative to female size, the eggs are large and the clutches are heavy, corresponding to approximately 27% of female body mass.

Key words: colubrid, fecundity, sexual dimorphism, snakes, reproduction

INTRODUCTION

The genus *Atractus* Wagler, 1828 comprises about 80 small fossorial species of snakes that occur in South America, from eastern Panama to western Argentina (Fernandes & Puerto, 1993; Fernandes, 1995; Giraudo, 2001). The reproductive biology of most species is poorly known, and available data are mainly from very small samples (e.g. Martins & Oliveira, 1993, 1998; Fernandes & Puerto, 1993; Murphy, 1997; Starace, 1998; Cassimiro *et al.*, 2002). *Atractus reticulatus* (Boulenger, 1885) corresponds to the southern distribution of the genus, occurring in southern Paraguay, north-eastern Argentina and south-eastern and southern Brazil (Fernandes, 1995; Giraudo & Scrocchi, 2000). This species inhabits forests, savannas, transitional environments and semi-urban areas (Giraudo & Scrocchi, 2000). The reproductive biology of *A. reticulatus* has been almost completely ignored. There is only anecdotal information on the incubation time of two clutches from south-eastern Brazil, and biometry of neonates from one of them (Fernandes & Puerto, 1993).

Herein we present data on sexual dimorphism in body size, sexual maturity, reproductive cycle, fecundity and recruitment of *Atractus reticulatus* in southern Brazil.

MATERIAL AND METHODS

STUDY AREA

All specimens examined were from eastern Depressão Central of Rio Grande do Sul State, southern Brazil. This area encompasses a zone of lowlands (10-300 m above sea level) with dark red podzol soil, giving rise to poorly drained land with high humidity. The relief is homogene-

ous, forming hillocks. Some disjunct hills are present and the presence of man is high in some areas. The climate is mesothermic temperate (IBGE, 1990).

MATERIAL EXAMINED

This study was based on the dissection of 135 specimens preserved in the collections of Museu de Ciências e Tecnologia of Pontifícia Universidade Católica do Rio Grande do Sul (MCP) and Museu de Ciências Naturais of Fundação Zoobotânica do Rio Grande do Sul (MCN) (see Appendix 1). Of these, three specimens were maintained alive and laid eggs in captivity, providing additional data on clutch size and time of oviposition.

PROCEDURES

The data recorded for each specimen were: snout-vent-length (SVL, mm), tail length (TL, mm), sex (analysis of gonads or hemipenis muscles), number of vitellogenic follicles or eggs, size of the largest vitellogenic follicle or egg (in mm), and condition of efferent ducts.

Females with follicles at least 6 mm in length or with eggs in the oviducts were considered mature (modified from Shine, 1977). Males with convoluted and opaque efferent ducts were considered mature (Shine, 1988). The age and size at maturation were estimated based on the seasonal size classes of males and females over time (as in Shine, 1988). This estimative is possible when reproduction (mainly hatchlings) is markedly seasonal. The size and sex of the neonates, and the time of hatchlings were recorded from eggs incubated artificially (see below). The time of maturation was considered to be the time interval between the hatchlings and the first appearance of mature males and females in the sample. Gravid females were kept in captivity until oviposition. Eggs were measured (largest

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length), weighed and incubated in vermiculite at about 25 °C. The relative clutch mass (RCM) was calculated by dividing the total clutch mass by the sum of female mass and total clutch mass. Only hatchlings hatched in captivity were considered as neonates. The degree of sexual size dimorphism (SSD) was the difference of the mean size (SVL) of the largest sex divided by the mean size of the smallest sex to 1.0 (Shine, 1993). Statistics were performed using SPSS for Windows v. 10. We employed a two-tailed Student's *t*-test to evaluate the statistical significance of sexual dimorphism.

RESULTS

BODY SIZE AND SEXUAL DIMORPHISM

Neonate SVL ranged from 77 mm to 117 mm (mean±SD = 107.7±12.9; *n* = 10) and TL from 5 mm to 17 mm (mean±SD = 13.5±3.5; *n* = 10). Mature female SVL ranged from 242 mm to 370 mm (mean±SD = 300.1±30.0; *n* = 43) and TL from 26 mm to 44 mm (mean±SD = 35.0±4.54; *n* = 43). Mature male SVL ranged from 198 mm to 295 mm (mean±SD = 249.9±24.4; *n* = 58) and TL from 26 mm to 51 mm (mean±SD = 38.2±5.8 *n* = 58). The SVL of mature females was significantly larger than the SVL of mature males (*t* = 1.98, *df* = 99, *P* < 0.001), and the TL of mature males was significantly larger than the TL of mature females (*t* = 1.98, *df* = 99, *P* < 0.001). The degree of sexual size dimorphism was calculated as 0.20.

SEXUAL MATURITY

The combined analysis of neonate body size, birth time and seasonal distribution of body size classes (SVL) suggests that males reach sexual maturity at eight to 10 months old (SVL of nearly 198 mm, Fig. 1) and females at 11 to 12 months old (SVL of nearly 242 mm, Fig. 2).

REPRODUCTIVE CYCLE

Vitellogenesis began in late August and lasted until late December (Fig. 3). Females were collected together with mature males in November (*n* = 2). Clutches were observed in November, December and January. Hatchlings were recorded from January to March (from three clutches). One female had vitellogenic follicles while carrying eggs in the oviducts. Another female was dissected immediately after laying eggs and also had vitellogenic follicles.

CLUTCH SIZE AND BIOMETRY OF EGGS AND NEWBORNS

Fecundity in *Atractus reticulatus* was low. The number of vitellogenic follicles varied from 2 to 6 (mean±SD = 3.9±1.5, *n* = 9), and the number of eggs varied from 1 to 3 (mean±SD = 2.7±0.8, *n* = 6). Within the six females that contained eggs, five females (measuring from 242 mm to 333 mm SVL) had three eggs, and one female (measuring 286 mm SVL) had one egg. Relative

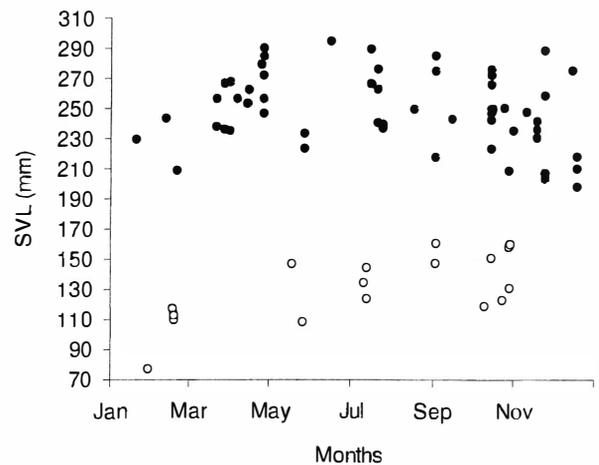


FIG. 1. Seasonal distribution of body size in males of *Atractus reticulatus* from southern Brazil. Open circles, immature males; closed circles, mature males.

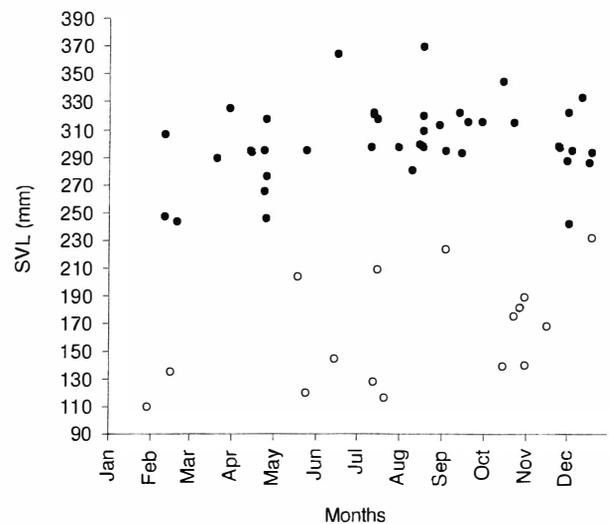


FIG. 2. Seasonal distribution of body size in females of *Atractus reticulatus* from southern Brazil. Open circles, immature females; closed circles, mature females.

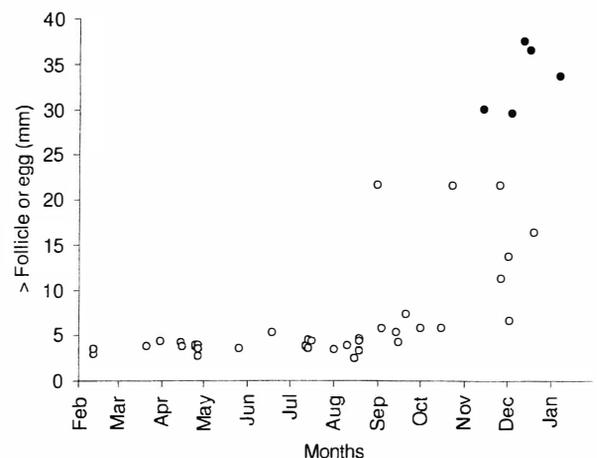


FIG. 3. Seasonal variation in the diameter of largest follicles (open circles) and eggs (closed circles) of mature females of *Atractus reticulatus* from southern Brazil.

to female size, the eggs are large (mean length \pm SD = 34.6 \pm 3.92 mm, mean mass \pm SD = 1.7 \pm 0.25 g, $n=10$), and the clutches are heavy, corresponding to approximately 27% of the female weight (calculated from means, $n=4$). After 66 days of artificial incubation, one clutch with three eggs produced three neonates measuring mean SVL \pm SD of 116.3 \pm 0.58 mm and mean \pm SD mass of 1.31 \pm 0.32 g.

DISCUSSION

BODY SIZE AND SEXUAL DIMORPHISM

The SVL of neonates of *Atractus reticulatus* is relatively large when compared to the SVL of conspecific adults, and also large when compared to the SVL of neonates of other Neotropical colubrids (Fernandes & Puerto, 1993). In snakes, female size and the number of eggs are usually correlated and this affects neonate size (Seigel & Ford, 1987). The small size of *Atractus reticulatus* and the low fecundity seem to be adaptations to the underground environment, as observed in other morphostructural characteristics of fossorial snakes (Cadle & Greene, 1993; Webb *et al.* 2000a, 2001). Lemen & Voris (1981) suggest that some small species of marine snakes (*Hydrophis torquatus*) may produce large offspring while reducing clutch size. Availability of food is higher to large neonates than to small neonates since the former can swallow larger prey. Madsen & Shine (1998, 2000) recorded mortality rates higher than usual among *Liasis fuscus* neonates that were born late and, hence, small in relation to their prey size.

Larger SVL in females corresponds to the most common pattern in snakes. It is recorded, as a rule, among species in which male-male combat does not occur (Shine, 1978, 1984). There is no record of male-male combat in *A. reticulatus* or in any other species of the genus, thus the pattern found here was as expected. Natural selection may also favour larger body size in females that incur high reproductive costs (Bonnet *et al.*, 1998). *Atractus reticulatus* seems to belong to this category since its clutches are small but comprise large and relatively heavy eggs when compared to female body size.

The sexual dimorphism in TL, with larger values in mature males than mature females, also corresponds to the most common pattern in snakes (King, 1989; Shine, 1993). Giraudo & Scrocchi (2000) analysed specimens of *Atractus reticulatus* combining all available sizes and ages and recorded sexual dimorphism in the number of ventrals (greater in males), subcaudals (greater in females), and in the ratio tail length / total body length (greater in males). Our data demonstrate sexual dimorphism in SVL (greater in females) and TL (greater in males) in mature specimens.

SEXUAL MATURITY

In snakes the energetic costs incurred while reaching sexual maturity seem to be smaller for males than for females with high reproductive costs (Shine, 1978). The

delayed sexual maturity in females of *Atractus reticulatus*, in relation to conspecific males, may have advantages by allowing females to reach a larger body size at time of reproduction, and to produce larger eggs and hatchlings. Due to their faster maturation, mature males are available in the population when the first females reach maturity, which may improve the reproductive success of the species. However, only neonates of *Atractus reticulatus* emerging at the beginning of the hatchling period (January) appear able to reach a similar SVL to mature individuals in the reproductive season subsequent to their birth. Late neonates (March) may remain immature until the second reproductive season, as recorded by Oliveira (2001) for the colubrid *Lystrophis dorbignyi* on the North Coast of Rio Grande do Sul - an area geographically close to this study.

REPRODUCTIVE CYCLE

In the study area, the reproduction of *Atractus reticulatus* is seasonal, with recruitment during the hotter months of the year. In south-eastern Brazil, Fernandes & Puerto (1993) recorded two clutches in January, which corresponds to the end of the oviposition period of this species in southern Brazil. In *Atractus pantosticus*, a typically subtropical species with a more northern distribution than *A. reticulatus*, the reproductive period is longer, with records of clutches from January to March (Fernandes & Puerto, 1993; Cassimiro *et al.*, 2002). Martins & Oliveira (1993, 1998) investigated the biology of eight species of *Atractus* (*A. alphonsehoguei*, *A. latifrons*, *A. major*, *A. poeppigi*, *A. schach*, *A. snethlageae*, *A. torquatus* and *A. trilineatus*) at low latitudes (Manaus city and adjacent regions), where the climate is typically tropical, and suggested aseasonal reproduction for two of them (*A. latifrons* and *A. torquatus*). The temperate climate in the southern region of Brazil seems to impose some physiological constraints that limit snake activity to the hotter months of the year, and this influences the natural history of the species. Thus, much of what is known for Brazilian snakes of tropical regions may not be useful as parameters for the same species (or closely related species) in southern Brazil (Di-Bernardo, 1998).

CLUTCH SIZE AND BIOMETRY OF EGGS AND NEONATES

The low fecundity of *Atractus reticulatus* may be related to the small size of adults (Shine, 1983, 1984), since the larger congeneric species, *A. major* and *A. torquatus*, produce larger clutches (Martins & Oliveira 1993, 1998). The fossorial habit of *Atractus reticulatus*, may also influence the number and shape of the eggs of each clutch. Clutches of *A. reticulatus* consist of long eggs, which probably limit the presence of additional eggs in the oviducts. The increase in width and decrease in length of eggs could allow accommodation of additional eggs with constant mass (Ford & Seigel, 1989).

However, wide eggs possibly restrain the locomotion of fossorial snakes inside galleries under the ground. Clutches with similar parameters to *Atractus reticulatus* (small and containing long eggs) seem to be common to other species of this genus (Martins & Oliveira 1993, 1998; Fernandes & Puerto, 1993; Murphy, 1997; Starace, 1998; Cassimiro *et al.*, 2002), and to reptiles of other genera with fossorial or semifossorial habits, even from phylogenetically distinct lineages such as *Tantilla* (Aldridge & Semlitsch, 1982; Marques & Puerto, 1998), and *Amphisbaenia* (*Monopeltis anchietae* and *Zygaspis quadrifrons*, Webb *et al.*, 2000b - but see Webb *et al.*, 2001 with regards to typhlopids from southern Africa). On the other hand, some non-fossorial colubrid snakes from southern Brazil, with SVL similar to that of *A. reticulatus*, may produce up to 13 eggs (e.g. *Liophis jaegeri*, Pontes & Di-Bernardo, 1988).

Fernandes & Puerto (1993) recorded mean length of neonate *Atractus reticulatus* similar to our records, but recorded hatchlings during a much longer period.

Although the fossorial lifestyle of *Atractus reticulatus* makes the study of live individuals challenging, we show that some interesting reproductive parameters can be accessed by dissecting preserved specimens. We propose that the fossorial lifestyle of *Atractus* drives its reproductive parameters, for instance, reducing the number of eggs and increasing their lengths, thus providing more mobility to the snake in the subterranean environment. However, additional research must be made to test the hypotheses proposed here. *A. reticulatus* is a common snake that can be found even in places of anthropogenic disturbance (e.g. the suburbs of large cities), and we suggest that it would make a good model to test such hypotheses.

ACKNOWLEDGMENTS

We are thankful to Moema Leitão de Araújo and Maria Lúcia Machado Alves for borrowing part of the material used in this study. We thank Lize Helena Cappellari, John Measey and David Gower for improving the manuscript and Roberto Baptista de Oliveira for helping with statistical analyses. MD is partially financed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Process CNPq # 300.164/00-9), and RLB has a Master fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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APPENDIX 1

SPECIMENS EXAMINED

Brazil. Rio Grande do Sul: Esteio: MCN 9438; Gravataí: MCN 2954, 3000; Porto Alegre: MCN 1015, MCP 1907, 4842, 5186, MCN 8083, 8084, 8570, MCP 9069, 9087, 9088, MCP 9318, 9388, 10129, 10130, 10131, 10209, 10210, 10211, 10212, 10509, 10510, 10515, 10965, 11258, 11259, 11356, 11901, 11903, 13178, 13179, 13180, 13281, 13299, 13300, 13363, 13364, 13365, 13382, 13383, 13384, 13385, 13386, 13407, 13408, 13409, 13410, 13463, 13464, 13465, 13466, 13527, 13528, 13529, 13530, 13531, 13532, 13533, 13534, 13716, 13792, 13793, 13794, 13795, 13803, 13804, 13805, 13977, 13978, 13979, 13980, 13981, 14121, 14179, 14234, 14235, 14236, 14237, 14238, 14280, 14295, 14303, 14304, 14305, 14306, 14307, 14393, 14398, 14399, 14402, 14403, 14433, 14434, 14435, 14436, 14437, 14438, 14439, 14440, 14441, 14471, 14472, 14473, 14474, 14570, 14596, 14597, 14598, 14599, 14600, 14601, 14639, 14640, 14641, 14642, 14643, 14644, 14645, 14646, 14647, 14675, 14676, 14697, 14698, 14699, 14700, 14701, 14702; Santo Antônio da Patrulha: MCP 4; São Leopoldo: MCN 7450; Taquara: MCP 11727; Viamão: MCP 8983.

SHORT NOTES

HERPETOLOGICAL JOURNAL, Vol. 15, pp. 201-203 (2005)

**INTERACTION OF THE STATIC NET
FISHERY WITH LOGGERHEAD SEA
TURTLES IN THE
MEDITERRANEAN: INSIGHTS FROM
MARK-RECAPTURE DATA**

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Comparison of mark-recapture data collected using different fishing methods suggests that in the Mediterranean the interaction of sea turtles with the static net fishery is very important and comparable to other fisheries. Given the high mortality rate observed in this and other studies, static nets are likely to represent a serious threat to Mediterranean sea turtle populations.

Key words: *Caretta caretta*, Mediterranean sea fishery interaction, static nets

Loggerhead sea turtles (*Caretta caretta*) are listed as Endangered in the IUCN Red List of threatened species (Hilton-Taylor, 2000). In the Mediterranean Sea, the probable population sub-structure and genetic isolation from the Atlantic – at least at the female level – (Laurent *et al.*, 1998), make loggerhead turtles particularly vulnerable to the serious threats affecting them in the basin. One of the most important threats is the mortality associated with interaction with fishing equipment (see Gerosa & Casale, 1999, for a review). Thus, it is of the utmost importance to improve our knowledge of the impact of fisheries on sea turtle populations.

Trawl nets and drifting longlines have been recognized as methods that capture thousands or tens of thousands of turtles in the Mediterranean (see Gerosa & Casale, 1999, for a review; Casale *et al.*, 2004; Casale *et al.*, in press), thus representing a serious threat in this basin as well as in other areas (e.g. National Research Council, 1990; NMFS, 2001). However, the possible impact of other fishing methods has not been adequately addressed, especially for artisanal and amateur fisheries that may use static nets (trammel and gill nets anchored to the sea bottom in shallow waters).

Typically, the net is put in place at sunset and retrieved the next morning, and this theoretically suggests

a high mortality rate, because captured turtles are likely to be forced underwater for an unsustainable period of time. In some Mediterranean countries, mortality induced by static nets was estimated through reports by – or inquires to – fishermen. Although this method potentially gives low-biased estimates, high mortality was reported in most cases: 77.7% (Balearic Islands, Spain; $n=45$; Carreras *et al.*, 2004); 94.4% (Corsica, France; $n=18$; Delaugerre, 1987); 53.7% (Continental France; $n=149$; Laurent, 1991); 54.9% (Croatia; $n=51$; Lazar & Tvrtkovic, 2003); while in Tunisia only 5.2% ($n=58$; Bradai, 1993). Such a high mortality rate may represent a serious threat to the populations if a large number of turtles is caught.

Catch rate per static net vessel is probably low. For instance, inquiries to fishermen provided estimates of 0.5-2.1 turtles per vessel per year in Tunisia (Bradai, 1993) and 0.17 turtles per vessel per month in the Balearic Islands, Spain (Carreras *et al.*, 2004). This represents another problem for the study of this interaction, because it hides the phenomenon if compared with other fishing methods that have much higher catch rates. However, even low catch rates can result in high numbers of captures if associated with a high number of vessels.

On the basis of inquiries to fishermen, about 920 turtles per year may be captured by static nets in Tunisia (calculated from Bradai, 1993) and about 200 in the Balearic Islands (Carreras *et al.*, 2004). It should be taken into account that being based on inquiries, these figures may be underestimates, while the opposite is improbable.

Apart from these specific cases, it is difficult to gain a reliable estimate at the Mediterranean scale, because this is an artisanal and amateur fishery comprising very small vessels dispersed along many small ports, and usually not included in the country register, making official statistics unreliable (Di Natale, 2002): a realistic census is therefore difficult to obtain. However, Di Natale (2002) estimates that artisanal fishing vessels in the 21 Mediterranean countries number more than 200,000. Of these, about half may be static netters, if the proportion found in some countries (Lamboeuf, 2000; Alarcón Urbistondo, 2001) can be generalised.

Our data were collected in the period 1981-2000 in the framework of a sea turtle tagging programme carried out in Italy in collaboration with professional fishermen (Argano, 1992). Turtles were originally incidentally captured by fishing methods, landed, and then tagged and released by project personnel with monel tags (style 49 or 681, National Band and Tag, Kentucky, USA), attached to front flippers. Tags provided a postal address and the words "reward premio remite". Except for the text on the tags, no particular emphasis was given to rewards for reporting recaptures, and rewards usually consisted of project T-shirts. Recaptures were reported by fishermen directly to personnel involved in the tagging programme (if operating in the area and known by the fisherman) or by mail. We classified fishing methods

TABLE 1. Proportion of recaptured turtles out of all the turtles captured in Italy by different fishing methods.

Fishing method	%	N
Longline	0.8	1095
Static net	6.3	95
Unspecified 'net'	3.3	153
Trawl net	3.0	296
Other	2.2	46
Unknown	5.6	72
Total	1.9	1757

as: drifting longline, bottom trawl, static net, unspecified net (these records reported just 'net', which can mean either trawl, drifting, or static net), other or unknown gears (Table 1).

We received reports of 105 turtles captured or recaptured by static net fishermen (collaborating or not with our programme) from Italy and other Mediterranean countries. Measured turtles ranged from 21 to 80 cm Curved Carapace Length notch-to-tip (mean = 45.8; SD=13.0; $n=70$). The condition was unknown for five of them and 11 specimens out of the other 100 died as a consequence of the capture. However, fishermen probably preferentially reported the capture of live specimens, either to avoid providing evidence of the impact of their activity on protected species, or because they believed dead specimens to be less interesting for our study. In this respect it is interesting that when fishermen had an additional reason to report a capture (i.e. a turtle with a tag), the proportion of dead turtles was higher (five out of 11) than among turtles without a tag (six out of 89; Fisher exact test; $P<0.005$; $n=100$). Hence, the mortality rate estimated from fishermen reports should be considered with caution, while the one based on recaptures only is expected to be more representative of the actual mortality. High mortality rates were reported by another mark-recapture programme too: of six tagged specimens found in Croatian static nets five were dead (Lazar *et al.*, 2000).

Recaptures of tagged turtles were probably underreported by Italian static net fishermen in compari-

TABLE 2. Percentage of turtles recaptured by different fishing methods in the Mediterranean. For Italy, recaptures reported by fishermen not directly involved in the project are also shown (see text).

	Italy All vessels ($n=34$)	Italy Non-collab. vessels ($n=19$)	Other countries ($n=14$)
Longline	26.5	0.0	0.0
Static net	17.6	31.6	57.1
Unspecified 'net'	14.7	26.3	21.4
Trawl net	26.5	15.8	7.1
Other	2.9	5.3	0.0
Unknown	11.8	21.1	14.3
Total	100.0	100.0	100.0

son to other countries, for two reasons: first, our research program focused particularly on longline and trawl, and second, Italian fishermen might prefer not to report to Italian authorities the death of a protected species (Argano *et al.*, 1992), which is often the case, given the high mortality rates above. Even so, static net Italian fishermen reported the highest proportion of recaptured turtles of the total number of reports (Table 1) and static nets are well represented among the fishing methods recapturing tagged specimens in Italy (Table 2). Furthermore, when recaptures reported by Italian fishermen directly involved in the project (working with longline and trawl) are excluded, so reducing the bias towards longline and trawl, static nets are the main fishing method by which tagged turtles were recaptured, as it is the case for recaptures from other countries unaffected by the suspected Italian biases (Table 2). A similar result was observed in Croatia, where static net fishermen reported six out of nine turtles recaptured (Lazar *et al.*, 2000).

Longline fishermen rarely take aboard a captured turtle (and so they cannot see a tag), and this may explain the low number of specimens recaptured by this method, known to interact with a large number of turtles (see Gerosa & Casale, 1999). On the other hand, both static net and trawl fishermen have the opportunity of a close look at the turtle. Hence, although alternative explanations cannot be excluded, the relative proportions of recaptures reported by trawl and static net fishermen suggest that in the Mediterranean the overall interaction between sea turtles and the static net fishery may be as important as – or even more important than – the interaction with the trawl fishery.

This scenario underscores the urgent need to obtain reliable estimates of fleet size in different countries, and related turtle catch and mortality rates, in order to assess the impact static nets have on the Mediterranean sea turtle populations.

Acknowledgements. We thank the many fishermen and all the persons who participated in the "Progetto Tartarughe" (Univ. Roma/WWF Italy).

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Accepted: 1.10.04

BERGMANN'S RULE IS SIZE-RELATED IN EUROPEAN NEWTS (*TRITURUS*)

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The validity of Bergmann's rule – which describes the intraspecific increase in body size with increasing altitude – was studied in several populations of three European newt species. There was a positive correlation between body size and altitude for smaller-bodied *Triturus vulgaris* and *T. alpestris* species, but no relationship between body size and altitude for the much larger sized *T. carnifex*. Body size therefore plays a role in the application of Bergmann's rule to European newts.

Key words: altitudinal gradient, morphology, *Triturus vulgaris*, *Triturus alpestris*, *Triturus carnifex*

Bergmann's rule states that there is an intraspecific tendency toward larger body size in cooler environments (Mayr, 1956). However, tests of Bergmann's rule have traditionally relied upon latitude or altitude as a proxy for temperature (Ashton *et al.*, 2000). Originally restricted to mammals and birds (Mayr, 1956), it was recently claimed that Bergmann's rule holds true for amphibians in general, but more clearly for tailed amphibians than for anurans (Ashton, 2002a). However, body size data (10 or more location sites) for several populations spread over altitude/latitude gradients are available for only a few species. For instance, among the European newts (*Triturus* spp.), only limited data for *T. alpestris* and *T. marmoratus* have been reported (Miaud *et al.*, 2000 and Díaz-Paniagua *et al.*, 1996, respectively). More studies, with extensive sampling, of body size variation of amphibians are required to further evaluate the validity of Bergmann's rule for this group.

We collected population body size data for three *Triturus* species to further evaluate Bergmann's rule in amphibians. The sampling locations vary considerably in altitude, but little in latitude (less than 5°). This enables a test of the intraspecific association between body size and altitude while minimizing the possible confounding effect of latitude. Because the three species of *Triturus* differ in body size (maximum total lengths for *T. vulgaris* and *T. alpestris* are about 100 mm, whereas it is about 170 mm for *T. carnifex*; Griffiths, 1996), we

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also investigated the influence of body size on intraspecific size-altitude body size trends.

Population samples came from Slovenia, Croatia, Bosnia and Herzegovina, Serbia and Montenegro and FYR Macedonia. The studied newts came from Dr Georg Dzukic's Herpetological Collection (Institute for Biological Research, Belgrade). Only mature newt individuals, collected during the breeding season, were studied. Due to the sexual dimorphism in newts, which is most prominent in *T. alpestris* (e.g. Kalezic *et al.*, 1992), males and females have been treated separately in our study. Our data set was obtained from 39 population samples of *T. vulgaris* (mean no. of specimens per sample ± SD: 22.0 ± 3.84 and 22.28 ± 3.68 for males and females, respectively), 19 population samples of *T. alpestris* (24.8 ± 5.3 males and 25.7 ± 5.3 females), and 22 population samples of *T. carnifex* (16.5 ± 3.8 males and 17.5 ± 3.4 females). Samples were collected from most of the vertical distributional range recorded for the study species (see Griffiths, 1996). The ranges were: 0-1650 m for *T. vulgaris*, 80-2100 m for *T. alpestris*, and 0-1550 m for *T. carnifex*.

The snout-vent length (SVL), distance from the snout to the posterior edge of the cloaca, was measured for each specimen from each population. Variation in body size within species, represented by the difference between the minimum and maximum average SVL values of the population samples studied, was as follows: *T. vulgaris* 30.1-43.1 mm and 30.3-44.7 mm (males and females, respectively); *T. alpestris* 37.7 - 48.2 mm in males and 42.5- 55.0 mm in females; *T. carnifex* 103.7-129.7 mm in males and 117.0-146.9 mm in females.

We used simple linear regression analyses to assess the relationship between body size (average SVL values for sampled populations) and altitude for each of the *Triturus* species. Average SVL was significantly positively associated with altitude for *T. vulgaris* ($r=0.44$, $P<0.01$ for males; $r=0.46$, $P<0.01$ for females), and for *T. alpestris* ($r=0.59$, $P<0.01$ for males; $r=0.53$, $P<0.05$ for females; Fig. 1). However, the larger-sized *T. carnifex* did not show a significant relationship between SVL and altitude for males ($r=0.010$, $P=0.96$) or females ($r=0.087$, $P=0.70$; Fig. 1).

To date, the relationship between body size and the tendency of a Bergmann's rule trend has been studied in birds and mammals. While no relationship between the tendency of a Bergmann's rule trend and body size has been found in birds (Ashton, 2002b; Meiri & Dayan, 2003), conflicting results have emerged for mammals (Ashton *et al.*, 2000 vs. Meiri & Dayan, 2003; Freckleton *et al.*, 2003). Apparently, Bergmann's rule appears to be size-related in European newts. In contrast to the much smaller *T. vulgaris* (this study) and *T. alpestris* (Miaud *et al.*, 2000; this study), large-bodied species (*T. marmoratus*, Díaz-Paniagua *et al.*, 1996 and *T. carnifex*, this study), do not show significant relationships between body size and altitude or latitude. Larger-bodied species may not show significant size trends because their large size makes them less sensitive

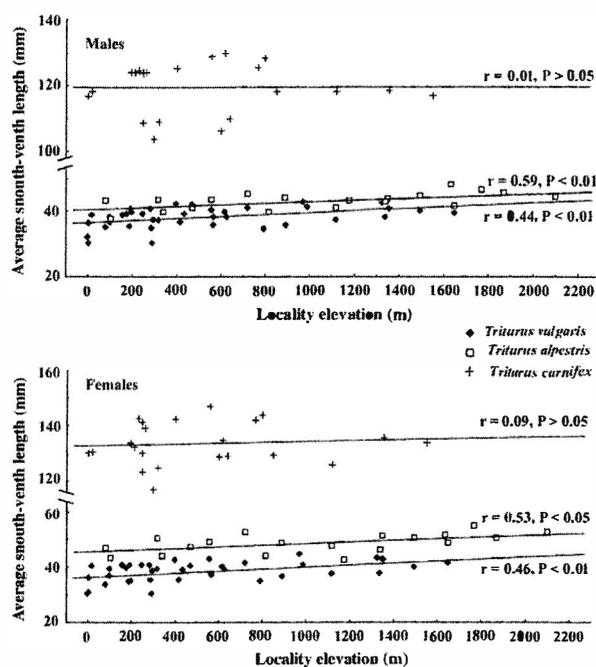


FIG 1. Relationship between body size (snout-vent length) and locality elevation for males and females of *Triturus vulgaris*, *T. alpestris* and *T. carnifex*.

to environmental differences across populations (e.g. temperature, water availability, difference in activity period). Together with selection acting on different life-history traits correlated with body size, such factors may underlie geographical size variation in amphibians (Ashton, 2002a; Morrison & Hero, 2003).

In conclusion, smaller-bodied species of European newts (*T. vulgaris* and *T. alpestris*) increase in body size with altitude, whereas body size for the larger-sized *T. carnifex* does not change with size, suggesting that body size of smaller-bodied salamanders is more influenced by environmental factors. Given that all three species are broadly sympatric over much of their altitudinal ranges, they represent promising candidates for further research aimed at examining mechanisms that generate patterns of body size variation in terrestrial ectothermic vertebrates.

Acknowledgements. Authors thank Lj. Tomovic and K. Ljubisavljevic for help during course of the study. The clarity of this article was greatly improved by comments from K. Ashton, C. Miaud, C. Morrison and an anonymous reviewer who also improved English and style. Serbian MSTD (project: "Integrative study of amphibians and reptiles of the Central Balkans", No. 1623) financed preparation of this paper.

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Accepted: 1.10.04

BOOK REVIEWS

Amphibians and Reptiles of Portugal, Madeira and the Azores-Archipelago. Malkmus, Rudolf (2004). Distribution and Natural History Notes. A.R.G. Gantner Verlag, Ruggell, Liechtenstein Pp. 448. ISBN 3-904144-89-8. Euros 80.00 (cloth).

The new book by Mr Rudolf Malkmus on the 'Amphibians and Reptiles of Portugal, Madeira and the Azores-Archipelago' brings to a splendid conclusion what is nothing less than a life's work. Malkmus relentlessly surveyed the amphibians and reptiles all over Portugal, over a period spanning nearly three decades, starting in 1976. This is one of the first national atlases of a continental European country to be published in the English language. Previous works by the same author on the Portuguese herpetofauna published in 1982 and 1995 are now superseded. In the first extensively illustrated chapter, the environment and landscape, climate and vegetation are described, followed by short accounts on the systematics, evolutionary history and palaeoherpetology of the Portuguese amphibians and reptiles. The natural history information is brief, but if more background information is required the reader may consult García-París *et al.* (2004) for amphibians. As before, the focus of presentation is on the distribution maps (17 for the amphibians and 31 for the reptiles). The maps make use of the 10×10 km UTM-grid system, with a 5×5 km scale representation of records, therewith doubling or tripling the information content of previous atlases (Malkmus, 1995; Godinho *et al.*, 1999), to over 15,000 observations. The information density is such that one may start to discover real distribution gaps, were previously such were mere interpretations (Ferrand de Almeida *et al.*, 2001). The reverse is also true; some ranges that appeared disconnected are now linked up (e.g. *Salamandra salamandra* and *Hyla meridionalis*). A word of caution is in place for the map projection over the UTM-grid that is not completely congruent to the earlier version. For example, the island of Berlenga, off the coast near Peniche, is currently situated at the bottom of UTM grid cell MD65 whereas it was positioned at the top of that grid cell previously (Malkmus, 1995). The consequences of this small inconsistency will hopefully be addressed in the production of the 'Atlas Nacional Herpetologia', a work undertaken by us in Porto at CIBIO and our colleagues at the University of Lisbon, under the administration and funding of the Portuguese Institute for Nature Conservation.

The printing and production of the hardbound volume is good. The book includes a large number of high quality colour photographs: about 80 full colour photographs of landscapes are well chosen and highly illustrative and nearly 200 attractive photographs of the species - about half of them made by the author - include the larvae of some amphibian species. What has not improved over the 1995 version is the referencing. I found a couple of flawed citations among the few that are in-

cluded in the main text. More importantly, most references are just listed at the end of the respective species chapters, which makes it difficult or impossible to know what statements stem from the personal knowledge of the author, or are derived from existing data. Finally, those who are familiar with the Portuguese situation know that the strong complaints about lack of care, outright neglect and wide-scale degradation of the landscape are by no means exaggerated.

This book is a must for anyone with an interest in the Iberian herpetofauna. The listed selling price of 80 euros is a fair price given the high quality production of the book. However, under the small margins for profit provided by the distributor and with the addition of shipping costs most bookshops will charge you around 110 euros.

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The Venomous Reptiles of the Western Hemisphere. Jonathan A. Campbell and William W. Lamar, with contributions by Edmund D. Brodie III, Edmund D. Brodie Jr., Ronald L. Gutberlet Jr., Michael B. Harvey, Robert Norris, David Warrell, and Vinícius Xavier Da Silva. (2004). Cornell University Press, Ithaca, New York, USA. Volume I: xviii+1476+28 pp. Volume II: xiv+477-870+28 pp. Hardcover. UK £86.95; US \$149.95. ISBN 0-8014-41-41-2 (cloth).

Of the many books published during the last few decades on venomous reptiles, perhaps none have been more widely acclaimed than *The Venomous Reptiles of Latin America*, by Jonathan Campbell and William Lamar (Cornell University Press, 1989). With its detailed coverage of all the dangerously harmful species known at the time from Latin America and the Caribbean, it was greeted with universal praise and is now regarded as something of a herpetological classic that

paved the way for many subsequent studies. This new book from Campbell and Lamar is essentially a revised and updated version of the first, but in covering a much broader geographical range – expanded to include the United States and Canada – it deals with a larger set of species (192), among them all of the rattlesnakes and an additional 14 species from Latin America described since 1989.

The Venomous Reptiles of the Western Hemisphere (hereafter VRWH) is published in two volumes, each of them strikingly presented with glossy dust jackets featuring magnificent photographs of a Langsdorff's coral snake (Vol. 1) and Central American bushmaster (Vol. 2). Volume 1 opens with a contents page, list of tables, preface, and introductory section on the overall scope of the book, including a reasoned statement on species concepts and explanation of why the authors have chosen to adopt certain taxonomies but not others. As in the original Latin American opus (VRLA), there then follows a series of regional accounts with information on the occurrence of species in particular countries, including keys to their identification. For most countries the keys are given in both English and Spanish (or Portuguese for Brazil), but in several notable cases they are not. Why Canada and the United States for example are provided with Spanish versions but not Guatemala, Honduras, Costa Rica and some of the other traditionally Spanish-speaking countries is a little puzzling. Descriptive accounts of the major taxonomic groups are then provided, beginning with the venomous lizards, coral snakes, and sea snakes, and continuing in the first part of Volume 2 with the pitvipers. Each of these sections are complimented with keys in both English and Spanish (also Portuguese for the coral snakes and species of *Bothrops*) and include a list of generic synonyms, etymology, characteristics, and an extensive summary of natural history with detailed information on activity, behaviour, conservation, prey and feeding ecology, parasites, predators, venom, reproduction, and longevity. Accounts of the species themselves include synonyms, lists of vernacular names, etymology, distribution, habitat, detailed information on lepidosis and colour pattern, comparisons to similar species, and a final 'Remarks' section with comments relating mostly to taxonomy. The remaining part of Volume 2 is comprised of equally authoritative sections on venomous snake mimicry (by E.D. Brodie III and E.D. Brodie Jr.), evolutionary relationships (R.L. Gutberlet Jr. and M.B. Harvey), venom poisoning by North American reptiles (R. Norris) and the features and treatment of snakebites in Central and South America (D. Warrell). A Glossary, Literature Cited section, and Index (usefully repeated in Volume 1) complete the work.

Both volumes are lavishly illustrated with a combined total of 1500 colour photographs. Most of these are of the venomous species described or their harmless mimics, but there are also a considerable number (135) showing the consequences of snakebite. Some of the images in the latter category are by their nature grue-

somely explicit, but they serve an important purpose in illustrating just how variable and unpredictable the effects of snakebite can be, and are also a graphic reminder of the kinds of injuries that some of these animals are capable of inflicting. Medical practitioners will find them especially useful in comparing the symptomatic features of bites from different species. There are also 161 black and white photographs, 109 ink drawings, eight full-page colour maps of physiography, topography, and vegetation, and 113 completely revised distribution maps. All of the illustrations are of excellent quality.

Advances in venomous snake systematics have resulted in many changes over recent years, none more so than with regard to the neotropical pitvipers. Several new genera have been erected (e.g. *Atropoides* for the jumping vipers and *Cerrophidion* for some of the terrestrial montane species, both previously contained within *Porthidium*) and a number of taxa previously considered as subspecies have been elevated to full species rank (e.g. the cantil *Agkistrodon taylori* and bushmasters *Lachesis melanocephala*, *L. muta* and *L. stenophrys*). Numerous other forms have been subsumed within synonymy. All of these changes have been implemented in VRWH, and the authors themselves also recognise a number of additional forms, including a fourth species of bushmaster from eastern Panama, *Lachesis acrochorda*, and two new tropical rattlesnakes in the *Crotalus durissus* complex, *C. simus* and *C. totonacus*. A conservative approach has been adopted throughout the book concerning the recognition of subspecies, but there is no consistency in their treatment within the text. While a full account of salient characters are provided for the subspecies of certain forms, for example (e.g. *Micrurus dumerili*, *Crotalus oregnaus*), only the basic details of distribution are provided for others (e.g. *Micrurus diastema*, *Crotalus simus*). Since the appearance of VRLA our understanding of relationships among venomous snakes from the Americas has increased considerably, but it is clear from reading through the accounts in VRWH that for many, particularly the coral snakes, a considerable amount of work still remains to be done. For some of the pitviper groups the taxonomic arrangement followed in VRWH is also unlikely to be the last word on the subject, a case in point being the recognition of *Bothriopsis* as a genus distinct from *Bothrops*.

An outstanding feature of VRWH is the remarkable depth of coverage provided in the taxonomic accounts, which together make up over half of the total page complement (95–475 in Vol. 1 and 477–616 in Vol. 2). For some species they extend over more than five pages (e.g. *Bothrops asper*, *Crotalus durissus*). All of the information is supported by numerous references and the author's own research or extensive field experience. Having accumulated a large data set over the years during the course of herpetofaunal studies in Belize, I subjected the descriptions of the eight species of dangerously venomous snakes known from this country to particular scrutiny, and found them to contain no con-

flicting information. Some of the comments were also revealing, particularly the identity of a pitviper specimen from west-central Cayo (USNM 61781), on which has hinged the disputed occurrence in Belize of *Porthidium yucatanicum*, a species otherwise known only from the northern part of the Yucatan Peninsula. Since its first citation by Schmidt (1941), USNM 61781 has been variously attributed to *Porthidium nasutum* and *P. yucatanicum*, but on the basis of its seven enlarged supralabials and the presence of a lacunolabial is, according to Campbell and Lamar, a misrepresented example of *Bothrops asper*. Of the genus *Micrurus* in Belize, the authors remark on the spurious distinction between *M. hippocrepis* and *M. diastema*, stating “were it not for the report by Gutiérrez *et al.* (1988) of differences in chromosomal morphology we would be inclined to consider them conspecific” (page 172). In respect of this it is interesting to note that the study by Gutiérrez *et al.* was restricted to material of *diastema* from Guatemala and did not include examples from near the range of *hippocrepis* in Belize, where specimens exhibit an intermediate (or at least similar) condition in having a reduced number of black bands (14–20 on body and 5–8 on tail) with little or no dark pigment on the red dorsal scales (pers. data). Notwithstanding the similarities noted by Gutiérrez *et al.* between *M. d. sapperi* and *M. d. apiatus*, it also seems reasonable to wonder if the various component populations of ‘*diastema*’ are as different from each other in karyotype as they are from *hippocrepis*. The authors’ inferred suspicion that *hippocrepis* may represent nothing more than another localised variant of *diastema* may well turn out to be true, but whatever its status the entire species group is clearly in need of further investigation and should benefit greatly from phylogenetic analysis using molecular data (currently in hand at the University of Texas, Arlington). The distribution of ‘*hippocrepis*’ in Belize appears to be restricted to the eastern versant of the Maya Mountains, not extending west of the divide as suggested by the map in VRWH; a possible example from Mountain Pine Ridge on the western side (MPM 7679), cited in Stafford (2000:81), has the usual low number of black body bands (11) but is somewhat anomalous in also having irregular black spotting on the red dorsal scales, and may instead represent an aberrant *diastema*. A further minor inaccuracy was detected also in the regional account of Belize, where the highest point is given as Victoria Peak (1122 m), but is in fact a little known ridge in the south of the country informally known as ‘Doyle’s Delight’ (1160 m).

As the largest and most exhaustive treatment of the subject available, VRWH is an essential reference for anyone involved in research on venomous reptiles of the Americas, or animals of medical importance in general. It deserves the highest recommendation and will almost certainly achieve the exalted status of its predecessor. Professional academics needing a comprehensive and up-to-date reference on the species of this region will find it especially useful, but with its

detailed information on natural history and snakebite it will undoubtedly appeal to many other potential users, from clinicians and field biologists to ecotourists and snake keeping enthusiasts. It is not without the occasional inaccuracy, but given its ambitious size and scope these are all relatively trivial and of little consequence. To everyone involved in the production of VRWH congratulations are in order for a phenomenal achievement.

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Incubation of Reptile Eggs. Gunther Köhler (Original German edition 1997, English edition 2005). Translated by Valerie Haecky. Krieger Publishing Company, Malabar, Florida, USA, 214 pp. ISBN 1-57524-193-5 US\$38.50 (cloth).

Eight years after his original German edition, Gunther Köhler has updated this book on incubation of reptile eggs and there has been an English translation by Valerie Haecky. This, and the American publisher, undoubtedly brings this work to a much wider audience. The result is an attractive hardback book that can be broadly split into three parts. The text is illustrated throughout with lots of colour photographs as well as half-tone photographs and line drawings.

The first part describes the biology of incubation of reptile eggs in both the natural situation and under artificial conditions. Hence, after a brief introduction there are chapters on egg morphology, egg development prior to laying and development of embryos up to and including hatching. A chapter is devoted to the “Physiological foundations of reptilian incubation”, i.e. temperature, humidity, gas exchange, egg movement and defence mechanisms against microbes. Unfortunately, some of this information – particularly for the last category – is lifted directly from that we know for birds and is not applicable to reptile eggs. Not that you would think this from reading the text, which rather misleads the uninitiated. A chapter then deals with natural nests and incubation. The remaining chapters in this

part of the book deal with maintenance of breeding reptiles in captivity and techniques for artificial incubation. There is a useful section on the various methods for building incubators, which will appeal to the hobbyist and researcher alike. Finally, various problems associated with development and incubation are discussed.

The second part is a series of descriptions of the specific incubation conditions for various types of reptiles as contributed by a variety of authors. These tend to be rather brief, partially through necessity given our general lack of understanding of reproduction in many species, but mainly because there are often no special conditions for specific reptile types. The resulting text is not very enlightening although it does tend to highlight specific scientific references for each type of reptile.

The last section, which forms a whole third of the page count, consists of three Appendices. The first is a brief and incomplete table on the effect of incubation temperature on sex determination in reptiles. We know so much more than what is presented – nowhere in the book does Köhler state that to date no snakes exhibit temperature-dependent sex determination. The second table gives details of the pattern of weight change for a small number of species. Again, so much more is known on this topic than is presented. The final table runs for around 50 pages and gives species accounts for clutch size, incubation temperatures and duration, together with the appropriate references. Unfortunately, this table has not been carefully prepared and checked in production so errors are common. For many species the information is limited to clutch size. As a result I find it hard to understand why this table is included. As a researcher I will find the list of references useful but the

data included in these Appendices would have been better presented by some kind of analysis that provided a general description of the incubation conditions for a particular family.

My initial impression of the book was quite favourable but reading it through I became uneasy about the content. The data presented seemed to be patchy in detail and accuracy, and at times there seemed to be a lot of emphasis on data presented from a review book published back in 1991 despite the wealth of information that has been collected since then. Köhler has made little attempt to bring together all of the available data and summarise it in a meaningful way. There is too much emphasis on presenting information without much explanation of what it means.

The target audience is probably the amateur herpetologist keeping and breeding pet reptiles but I feel the errors and the omissions in the text mean that this was a lost opportunity to bring good scientific understanding to a wider audience. If the book is aimed at the professional herpetologist then it fails to deliver a coherent review of available knowledge. People like myself are forced to revert to the original source references to ensure that the data are presented correctly. I am forced to conclude that whilst *Incubation of Reptile Eggs* is an attractive book and its main appeal is to those people breeding reptiles as a hobby, it could have been a lot better.

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