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FRONT COVER: *Scaphiophryne marmorata* (C. Raxworthy)

AUDITORY TUNING OF THE IBERIAN MIDWIFE TOAD, *ALYTES CISTERNASII*

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The study of auditory tuning in anuran amphibians is useful for understanding their reproductive behaviour. Auditory tuning is known for a relatively large number of anuran species but most of those studied are recently-derived species, rather than ancient. For one of the ancient species analysed, the common midwife toad (*Alytes obstetricans*), an unusual mismatch was found between the dominant frequency in the advertisement call and the tuning of the inner-ear organ that responds to the high frequencies characterizing the call (the basilar papilla). In this paper, the auditory tuning of a closely related species, the Iberian midwife toad (*Alytes cisternasii*), is analysed and the results are discussed in relation to behavioural experiments performed on this species. The results indicate that in *Alytes cisternasii* basilar papilla tuning closely matches the peak frequency in the call, as is common for recently-derived anurans. Furthermore, the tuning is consistent with behavioural measures of call preferences in this species.

Key words: anuran communication, mating behaviour, neurophysiology, vocalization

INTRODUCTION

Calls play a fundamental role in anuran communication in both male-male acoustic competition and female choice. This dependence has provided two effective means by which anuran reproductive behaviour can be studied: playback tests and neurophysiological experiments. The former reveal the behaviours of males or females when exposed to advertisement calls, whereas the latter generally reveal the response of the auditory system when exposed to sounds of different frequencies. In many cases these approaches have provided results that are consistent with each other. Neurophysiology studies have shown the anuran auditory system to be sensitive to frequencies emphasized in conspecific calls (Capranica, 1965; Zakon & Wilczynski, 1988; Gerhardt & Schwartz, 2001), and to indicate frequencies to which both males and females do in fact respond preferentially in playback studies (Capranica, 1965; Ryan *et al.*, 1990; Ryan *et al.*, 1992).

In anurans, the sensitivity to relatively low frequencies is mediated by the amphibian papilla (AP), one of the two inner-ear organs that amphibians possess, whereas sensitivity to relatively high frequencies is mediated by the basilar papilla (BP), the other inner ear organ (see reviews in Zakon & Wilczynski, 1988; Lewis & Narins, 1999). Different anuran species may have calls that stimulate both papillae, or may stimulate either one. It is generally accepted that there is a reasonably good match between the auditory tuning of the inner-ear organ stimulated by the conspecific advertisement call and the band or bands of frequency emphasized in the call (see review in Gerhardt & Schwartz, 2001). However, there may be small, but significant, mismatches

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between average tuning and the average advertisement call dominant frequency. Females of several anuran species have shown preferences for calls of lower than average frequency, which are usually related to larger male sizes (Ryan, 1988). For example, male midwife toads show non-random mating success (Márquez, 1993; Raxworthy, 1990), with larger males usually fertilizing more clutches. In addition, for at least three of the four species of *Alytes*, larger males emit advertisement calls with lower frequencies (Márquez & Bosch, 1995; Bush, 1997), and females are preferentially attracted to those lower frequency calls (Márquez, 1995*a,b*). Neurophysiology studies in the cricket frog (*Acris crepitans*) and the tungara frog (*Physalaemus pustulosus*) have shown that small mismatches between frequency tuning of the females' BP and male call frequency can potentially mediate mate choice for lower frequency calls, and thus directional sexual selection (Ryan *et al.*, 1990, 1992). Therefore, the study of auditory tuning can provide excellent clues to explain the non-random mating success of males frequently observed in nature and the resultant patterns of sexual selection.

Although the auditory tuning of a moderately large number of anuran species has been analysed to date, most of the approximately 25 species studied in any detail are recently-derived species rather than ancient (for reviews see Gerhardt, 1988; Walkowiak, 1988; Feng *et al.*, 1990; Fox, 1995; Gerhardt & Schwartz, 2001). Therefore, our understanding of how calls and tuning relate to each other is based mainly on recently-derived anurans. Wever (1985) used inner ear microphonic potentials to characterize tuning in several ancient amphibians (*Ascaphus truei, Leiopelma hochstetteri, Rhinophrynus dorsalis,* three *Bombina* species, and three pipids: *Xenopus laevis, X.borealis* and *Pipa pipa*), but did not discuss the relationship of tuning and calls in these species. In only two genera of anurans at the basal

level of amphibians, *Bombina* (*B. variegata*, *B. orientalis*, and *B. bombina*) and *Alytes* (*A. obstetricans*), has the relationship of calls and tuning been addressed (Mohneke & Schneider, 1979; Walkowiak, 1980, 1988; see also Wever, 1985, for tuning data in *Bombina*). Findings in *Bombina* and *A. obstetricans* are inconsistent. Mohneke & Schneider (1979) found a BP best excitatory frequency (BEF) of 1800 Hz. This value is very far above the mean call frequency known for this species (around 1280 Hz in Spanish populations; Márquez & Bosch, 1995) and, more importantly, this mismatch is in the opposite direction to the observed female preference for calls of lower than average frequency (Márquez, 1995a,b). It is thus opposite to the expected deviation that could explain directional sexual selection to low frequency calls. In contrast, in *B. variegata* there is a good match between frequency tuning of the BP and the animal's call frequency (Mohneke & Schneider, 1979).

Since the mismatch found in *A. obstetricans* by Mohneke & Schneider (1979) between frequency tuning of the BP and call frequency is unusual, in this paper we looked at the closely related species *A. cisternasii* to see if tuning far above the dominant call is characteristic of this genus. The Iberian midwife toad, *A. cisternasii*, is one of the three species of midwife toad that occur in the Iberian Peninsula, where it is confined to the southwestern quarter. In this species, males emit very simple tonal advertisement calls, which overlap substantially with *A. obstetricans* calls in frequency and duration (Márquez & Bosch, 1995; Fig. 1). In some areas, the two species are sympatric (see Márquez & Bosch, 1997, for a distribution map within the Iberian Peninsula). In addition to reporting neurophysiological tuning of the auditory system in *A. cisternasii*, we discuss the results in the context of the existing data on sexual selection for this species.

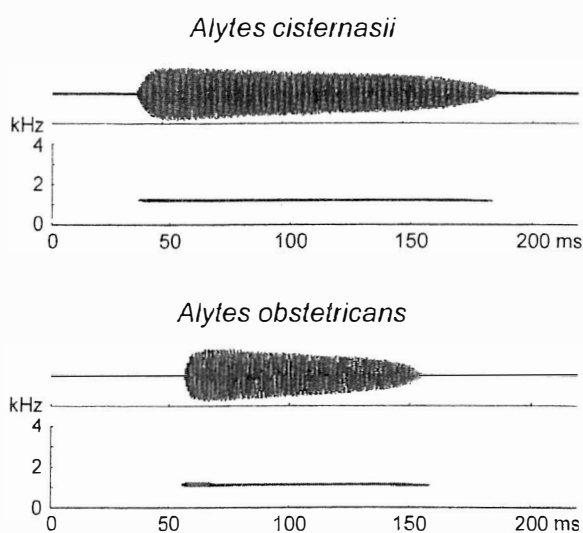### Alytes cisternasii

Alytes obstetricans

FIG. 1. Oscillograms (above) and audiospectrograms (below) of characteristic male advertisement calls of *A. cisternasii* and *A. obstetricans* from the studied sympatric population (see Márquez & Bosch, 1997 for details).

METHODS

Ten adults of *A. cisternasii* were collected in January 1998 in Aliste (Zamora, West Northern Spain), from a population sympatric with *A. obstetricans*. The capture took place outside the breeding season, and the animals were transported alive to the University of Texas at Austin, USA, and maintained in captivity. Both the animal room and the room in which the neurophysiology experiments were done were kept at 21°C.

Neurophysiological experiments were performed between May 1998 and August 1999 using methods previously used successfully with other small anurans (Ryan *et al.* 1990; Wilczynski *et al.*, 1993; Wilczynski *et al.*, 2001). All procedures were approved by The University of Texas Institutional Animal Care and Use Committee.

Animals were anaesthetized by immersion in a 2.5% aqueous solution of urethane. The midbrain was exposed by opening a skin flap on the top of the head and drilling a hole in the skull. The underlying meninges were then carefully removed. The surgical opening was then covered with a small piece of tissue paper, the skin flap was repositioned, and the animal was allowed to recover from the surgery for 1-2 days.

Following recovery, the animal was immobilized with an intramuscular injection of d-turbo-curarine chloride dissolved in a 20 mg/ml aqueous solution. The drug dose was approximately 10 µg/g body mass. The surgical opening was swabbed with lidocaine and the skin flap retracted to re-expose the dorsal surface of the midbrain. A drawn-glass micropipette filled with 3M KCl was placed into the midbrain and moved down into the torus semicircularis (inferior colliculus) using a Kopf hydraulic microdrive. Multi-unit auditory activity was isolated using a multi-tone search stimulus delivered to the ear contralateral to the recording site via a custom-made earphone assembly. Evoked auditory activity was monitored visually on a storage oscilloscope and acoustically using stereo earphones. With Wavetek stimulus generators and resistive attenuators, stimulus frequency and amplitude were controlled manually. Custom-made stimulus-control hardware was used to shape stimulus duration, rise/fall time, and repetition rate. Sound amplitude delivered through the earphone was calibrated with a B&K 2300 precision digital sound pressure meter at the end of a recording session by sealing the end of the earphone assembly over the meter microphone.

Once the electrode reached a location where robust evoked auditory activity was isolated, midbrain auditory thresholds were determined to frequencies from 100 to 4000 Hz in 100 Hz steps using 150 ms tone bursts repeated every 1.5 s. Threshold was defined as the minimum sound amplitude which could evoke discernible multi-unit neural activity. Once these thresholds were obtained, the process was repeated in smaller frequency and amplitude steps to obtain a more precise estimate of the best excitatory frequencies (BEF; fre-

quency with lowest threshold) of the two sensitivity peaks apparent in the recordings: the lower one we presume represents amphibian papilla tuning and the higher one we presume represents basilar papilla tuning (Wilczynski & Capranica, 1984). The procedure was repeated at three to six different electrode positions within the midbrain. Means were calculated for each animal based on dB values without converting to absolute pressure. Recordings at different positions sometimes resulted in different thresholds, but gave no differences in the shape of the audiogram or the positions of the threshold minima. This is probably due to the fact that the low impedance electrodes used for obtaining the multi-unit recordings summed activity over an area that was large relative to the small size of the toad midbrain, and is also consistent with previous reports indicating little or no tonotopic organization apparent in neurophysiological investigations of the anuran midbrain (Wilczynski & Capranica, 1984).

Neurophysiological experiments were completed successfully only on five animals. All the experimental animals were immediately euthanized by an overdose of the anesthetic MS222 (tricaine methyl sulfonate), either at the end of the neurophysiological experiment or when we could not obtain consistent, clearly recognizable, auditory-evoked activity above the background activity during the recording session. Snout-vent length was measured and sex was determined by dissecting the animal and looking at its gonads.

RESULTS

The five animals analysed were found to be males. Their snout-vent length (SVL) ranged from 31 to 37.9

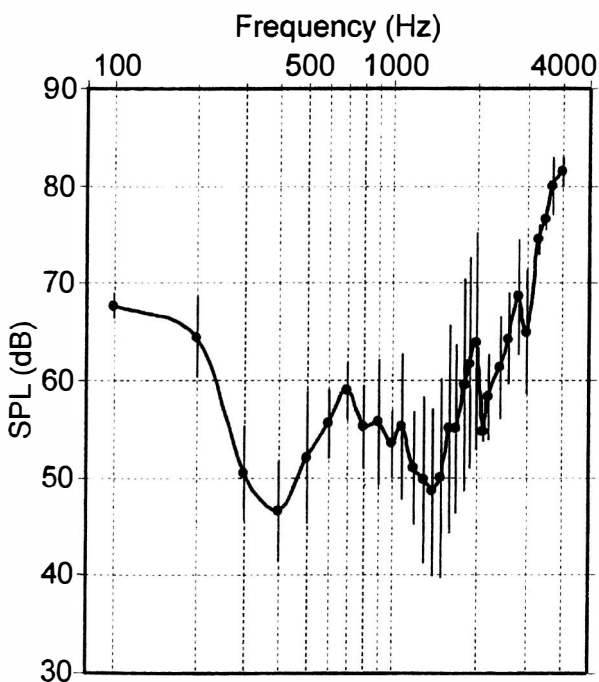
FIG. 2. Average audiogram (mean ± SE) for five *A. cisternasii* males. The average AP BEF is 417 Hz, while the average BP BEF is 1365 Hz.

mm (average 34.7 mm), slightly below the population average size (38.8±3.1 mm; Márquez & Bosch, 1997).

The average audiogram for the five analysed animals appears in Fig. 2. As expected, the tuning curve shows a bimodal pattern. We presume that the area of tuning from 100 to 1000 Hz represents the sensitivity profile of the amphibian papilla (AP). The second band of increased sensitivity, 1000-2000 Hz, represents the sensitivity profile of the basilar papilla (BP). In the five animals tested, the average AP BEF was 417 Hz, while the average BP BEF was 1365 Hz.

DISCUSSION

The bimodal appearance of the audiogram obtained for *A. cisternasii* is typical for neurophysiological recordings of auditory sensitivity in the anuran midbrain (Wilczynski *et al.*, 1984, 1993, 2001; Gerhardt & Schwartz, 2001). The two areas of frequency sensitivity have been shown to represent the contributions from the two inner-ear auditory organs, the lower frequency amphibian papilla (AP) and the higher frequency basilar papilla (BP). The area of greatest sensitivity, or best excitatory frequency (BEF), obtained for the BP matched the average male call dominant frequency for the population (1328±64 Hz; Márquez & Bosch, 1997). The BEF obtained for the AP confirms that this inner-ear organ in *A. cisternasii* is not likely to be involved in the recognition of acoustic species-specific signals, being related to detecting low frequencies not present in the conspecific call. Several small, recently-derived anurans (Neobatrachia) show the same pattern of specialization of the inner-ear organs seen in the present study for this more ancient species (Narins & Capranica, 1976; Wilczynski *et al.*, 1984, 1993; Ryan *et al.*, 1992).

Although the sample size for these experiments is not very high, the consistent and expected bimodal tuning curve obtained indicates reasonable accuracy for the purpose of this study. Furthermore, the studied species does not present the problem of having a call with a broad-band spectrum with substantial acoustic energy spars which characterizes many anuran advertisement calls. Instead, the total power in the *A. cisternasii* call is concentrated in a narrow frequency band with no harmonics and no frequency modulation.

The BEF obtained for the BP indicates that males in the studied population are matched or tuned slightly higher in frequency than the call. This is consistent with the majority of studies in recently-derived anurans (Neobatrachia) showing that BP tuning roughly matches call frequency. The small difference between BP BEF and call frequency found here is within the methodological sample error both in the acoustical analysis of male call frequency and the neurophysiological experiments. In fact, the standard deviation for the dominant frequency of the population is 64 Hz (over a sample size of 16 males recorded; Márquez & Bosch, 1997), double the difference found here. Therefore we are confident that there is no major mismatch between auditory tuning and call frequency in males of this species.

Although the results obtained here are only for males, in this species we can tentatively extend the conclusions to females. Despite the existence of good evidence for sexual differences in tuning, mediated in large part by size differences in other species (see Narins & Capranica, 1976; Wilczynski, 1986; Keddy-Hector, Wilczynski & Ryan, 1992; Wilczynski *et al.*, 1992), the sexes in *A. cisternasii* are similar in size, contrary to most anuran species. In typical habitat (i.e. Mérida, Extremadura, West Central Spain), the average SVL of males is 37.8 mm while that of females is 39.8 mm (Bosch & Márquez, 1996). Additionally, Mohneke & Schneider (1979) did not find significant differences between sexes in auditory tuning in *A. obstetricans*, although size ranges of males and females did not overlap. It will be necessary to obtain neurophysiology data from females before definitive conclusions can be reached, but given the data we obtained and extrapolations from the literature on other anuran species, it is very unlikely that females would be tuned greatly above males and therefore unlikely that they are tuned far above the conspecific call.

Our results contrast with those obtained by Mohneke & Schneider (1979), who found a considerable mismatch between the BEF of the BP and the dominant frequency for *A. obstetricans* advertisement calls. Since the animals they tested ranged from 36.5 mm to 45.5 mm, it is difficult to assume that the difference found occurred because the studied animals were significantly smaller than the average in their population. It is not clear from either the behaviour or the environmental conditions of *A. obstetricans* why that species should have such a great mismatch.

The closeness of BP tuning to the call dominant found here in *A. cisternasii* suggests that males from the studied population would be most sensitive, and presumably respond preferentially, to average calls from their own population. If females from this population were tuned about the same as males, they too would prefer average calls. This hypothesis is consistent with the behavioural experiments performed with females from this population by Márquez & Bosch (1997). Female preferences for calls with lower than average frequency have been found in several other populations of midwife toads (Márquez, 1995a,b), but not in females from the sympatric population whose animals we used for the present study. This could be because in the sympatric populations choosing a low frequency, the call may result in a heterospecific male being chosen (i.e. *A. obstetricans*), whose males produce a call with a slightly lower dominant frequency than that of the *A. cisternasii* call. This possibility should exert selection pressure against preferring lower frequency calls (Márquez & Bosch, 1997). Therefore, under this consideration, we would not expect to find a BEF lower than the average of male call frequency in the studied population. The behavioural results showing that preferences for low frequency calls occur in other, allopatric populations of

A. cisternasii suggest that there may be interesting population-level differences in the tuning of the auditory system in this species.

Further neurophysiological studies to determine whether *A. cisternasii* frequency-tuning is lower in allopatric populations than in sympatric populations could elucidate whether auditory tuning perfectly explains female *Alytes* preferences. Furthermore, additional studies of the auditory systems of other ancient anurans would be helpful in understanding whether the close relationship between calls and auditory tuning is as much a characteristic of basal anurans as it is of recently-derived taxa.

ACKNOWLEDGEMENTS

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DEMOGRAPHIC PROCESSES IN A SMALL, ISOLATED POPULATION OF NATTERJACK TOADS (*BUFO CALAMITA*) IN SOUTHERN BELGIUM

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In a small, isolated population of the natterjack toad in southern Belgium, some features of demography and reproductive behaviour were atypical. Fecundity (about 2000 eggs per clutch) and adult survival rate (27%, compared with typical values of 50-80%) were both very low. Population age-structure was skewed towards young toad (2-4 years) and male reproductive success was apparently independent of calling activity and the length of chorus attendance. These characteristics are interpreted in terms of isolation and small population size, and could indicate that the population is under stress.

Key words: mark-recapture, mating strategy, pond-fidelity, satellite tactic

INTRODUCTION

In the northern part of its range, the natterjack toad (*Bufo calamita* Laurenti) is adapted to habitats of early successional stages, such as heaths, dunes and alluvial areas. As such habitats are shrinking everywhere in Western Europe, the natterjack is more and more restricted to secondary habitats like slag heaps, gravel pits, sand pits and industrial ponds. Most of the 70 or so populations remaining in southern Belgium occur in such secondary habitats, seven of them being totally isolated (Percsy *et al.*, 1997; Percsy *et al.*, unpubl.).

In this paper, we report on the functioning of one such isolated population living in a former sand pit. We highlight the very small area used by calling males and we hypothesize a high frequency of satellite males (silent males attempting to intercept females attracted by the callers: Arak, 1988) as a consequence of the small area available for calling activity. Therefore, we focus on demographic parameters within this particular population (population density, sex-ratio and age-structure, individual survival and reproductive success) and their variations, compared to similar parameters from other populations (Beebee, 1979). We also investigate the consequences of the satellite parasitic behaviour for male reproductive tactics and success, as such parameters are sensitive to caller density (Arak, 1988; Tejedo, 1992*a*)

MATERIALS AND METHODS

THE SPECIES

The natterjack toad is a pioneer species adapted to living in unstable environments (Beebee, 1983). Its thermal requirements and susceptibility to predators and competitors in the aquatic stages often force the species to choose ephemeral ponds for reproduction (Andrén & Nilson, 1985*a,b*; Banks & Beebee, 1987; Griffiths, Edgar & Wong, 1991; Bardsley & Beebee, 1998; Denton & Beebee, 1997). Several demographic and behavioural adaptations have allowed the natterjack to use

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such habitats. The burrowing capacities of juveniles and adults allow them to use unvegetated terrestrial habitats (Beebee, 1983; Denton & Beebee, 1994). An extended breeding period (males assemble in chorus for 6-10 weeks) and the plasticity of larval growth help natterjacks cope with the risks of larval mortality due to pond desiccation (Beebee, 1983; Griffiths, 1997; Stephan *et al.*, 2001). A high female fecundity combined with high adult survival allows populations to recover rapidly, even after complete breeding failure (Beebee *et al.*, 1982).

STUDY SYSTEM

The study was performed from April to June in 1999 and 2000 in the former sandpit of Mellery (50°34'30" N, 4°34'20" E), southern Belgium. Frequent night visits around the site in search of calling natterjacks revealed that the population of Mellery is isolated from other breeding sites by a distance of at least 10 km. In Mellery, two ponds, separated by 250 m and differing both in area and the persistence of water, were used by calling males and spawning females during the duration of the study. The aquatic part of the habitat consisted of (1) the shallower parts (about 0.12 ha total) of a small permanent lake, and (2) the ephemeral waters (for an area of aquatic habitat ranging from 0 to 0.15 ha) of an artificially-built concrete pond. Terrestrial habitat surrounding the two ponds consisted mainly of (1) intensive agricultural fields and (2) an intensive clover area on a geotextile sheet covering the ancient quarry. These were interspersed with wooded areas and agricultural roads. The total area of suitable habitats (terrestrial and aquatic) for the toads and tadpoles was found to be 2 ha and 1 ha, respectively, according to the method of Denton & Beebee (1993*b*).

ESTIMATE OF POPULATION SIZE

Male total population size was derived from chorus size and composition, studied by MRR (Mark-Release-Recapture). Breeding activity of male natterjacks was monitored by visiting the breeding ponds at night, at least once each week, for a minimum of 2 hrs. Toads

were located by torchlight. Adult natterjacks were individually marked using a unique toe-clip code in 1999 or a passive integrated transponder (model TX1400, FishEagle Co., Lechlade, Gloucestershire, GL7 3QQ, England; Sinsch, 1992) in 2000. Wounds were treated with an antiseptic powder (Sulfamed, Medgenix). No formal estimate of chorus size was made in 1999 in the concrete pond because the pond dried up for several weeks during the breeding season. The number of males in 1999 is thus a minimal assessment rather than a total population estimate. During the MRR study, natterjacks were sexed and males were measured snout-to-vent (to the nearest 0.1mm) and weighed (to the nearest 0.5 g). Because toads tried to crouch when measured, we gently pressed them as flat as possible and each individual was measured twice at each capture event. Only the greatest length recorded was considered for further analyses. During handling all toads emptied their bladders, so the state of hydration was similar in all individuals. The first mass record was used for analyses.

Because no individual was found to switch between breeding ponds during one breeding season, MRR data for the two breeding ponds were analysed separately and the sizes of the two breeding assemblages were summed to give the size of the total male population. Since a given male could join and leave one chorus whenever he wanted, we consider this sampling unit to be an open system, and therefore MRR data were analysed using the Jolly-Seber (J-S) method as applied on open populations (Seber, 1982; Hines, 1988). Since all males usually participate in breeding activity every year (Sinsch & Seidel, 1995), a simple mathematical manipulation of the J-S results gives an estimate of the total number of males in each chorus. The estimate of N_1, the initial chorus size, is given by the ratio r_1/p where r_1 is the number of toads caught at the first date and p is the mean capture probability, calculated by the J-S method. The J-S method also gives a quantitative estimate of toad recruitment for each capture event (B_i), so we added the B_i's to the initial chorus size to estimate the total male population size in each breeding unit. The chorus sizes of each capture event were estimated using Jolly® (Hines, 1988).

MRR methods cannot be applied to females because they only stay one night in the pond to reproduce. We therefore used the census of egg clutches to assess the number of females. As other studies have shown that about 65% of female natterjacks reproduce every year (Tejedo, 1992c; Denton & Beebee, 1993a; Stephan *et al.*, 2001) and double clutching is very rare (Tejedo, 1992c; Denton & Beebee, 1993a; Sinsch & Seidel, 1995; Denton & Beebee, 1996), we estimated the total female population by dividing the clutch census by 0.65.

SURVEY OF REPRODUCTIVE ACTIVITIES

In order to estimate the fecundity of females, the two sites were visited during the day every 3-4 days to search for spawn strings. Recently laid clutches were carefully taken out of water, placed in a flat, white container filled with pond water, and photographed so that eggs counts could be made from slides later on. Clutches were immediately replaced in the original location.

In 1999, the reproductive activity of each male was evaluated by (1) his arrival date in the chorus and (2) his chorus attendance. In this study, chorus attendance corresponds to the percentage of time spent in the chorus: i.e. the number of days between the first and the last capture of an individual (his minimum residence time) divided by the total length of the breeding season. In 2000, a third estimate of reproductive activity was recorded: calling activity. Each time a toad was located during the MRR study of 2000, his behaviour (calling or silent) was recorded. Calling activity is the proportion of calling behaviour for toads for which at least three observations were recorded.

In 2000, we investigated the reproductive success of males: besides the MRR study, we also visited the site every night in search of toads in amplexus. Marked males were then identified. Reproductive success is the number of females with which a male was found in amplexus during the whole season.

SKELETOCHRONOLOGY

The age of 34 toads toe-clipped in 1999 was studied by skeletochronology. Removed phalanges were stored in 70% ethanol until they were cleaned, fixed, sliced and coloured with Cresyl violet (0.05%) according to skeletochronological methods used by Friedl & Klump (1997), Sinsch (1997) and Tejedo *et al.* (1997). Slides were observed under a light microscope and growth rings were counted in order to age the toads. In southern Belgium, as in Britain and Germany, growth slows down during winter. Observations of the bone structure therefore reveal dense and light-coloured rings corresponding to winter and summer growth, respectively.

ANALYSES

Statistical analyses were carried out using SAS® (SAS Institute, 1990): "PROC UNIVARIATE" was used to test the distribution of all parameters. Morphological variables (mass and length), their ratio for the 55 to 60 mm size-class, and fecundity each showed a normal distribution. To analyse the between-year differences in mean length of males, or female fecundity, we used a *t*-test after testing for equality of variances (SAS®: PROC TTEST). When variances were not equal between groups, or when there were significant departures from normality (for example, behavioural attributes of males and reproductive success), we compared groups using a Kruskal-Wallis test (Chi-square approximation) on simple, linear rank statistics based on the Wilcoxon scores (SAS®: PROC NPAR1WAY). We used Kendall's τ when testing for correlation between variables on an ordinal scale, such as mass, length, age, arrival date, chorus attendance and calling activity. Multiple regressions were used to detect

the influence of morphological and behavioural attributes on chorus attendance or calling activity (in 2000 only). Logistic regressions of males' reproductive success on five potentially explanatory variables (mass, length, arrival date, chorus attendance and calling activity) were then used to detect the influence of these individual variables on reproductive success in 2000.

RESULTS

DEMOGRAPHY AND MORPHOMETRY

Both chorus composition and chorus size fluctuated during the season, and the total size of the male population in one breeding assemblage exceeded the maximal daily size of the chorus. In 1999, the male population size of the lake was estimated at 56 males, and we marked nine other males in the concrete pond. Minimum male population size in 1999 was therefore estimated at 65 individuals. For 2000, MRR data gave an estimate of the total male population of 98 individuals. During 1999, 22 clutches were laid at Mellery, while in 2000 we counted 51. Adult breeding population size, calculated following the method described above, was estimated at 98 individuals in 1999 (minimal assessment) and 176 in 2000.

Accurate estimation of density relies on precise measures of suitable habitat areas used by toads. In the particular case of the Mellery population, these measures are facilitated by the fact that the three terrestrial habitat types surrounding both sites (i.e. agricultural fields, frequently-mowed clover on a geotextile substrate, and woodland) are totally unsuitable for natterjacks. This was confirmed by frequent, fruitless searching for adult toads by night with electric torches around the two sites, according to the method of Denton & Beebee (1993b). Thus, adult density was found to be 33 individuals per ha of habitat in 1999 (minimal assessment) and 59 individuals per ha of habitat in 2000.

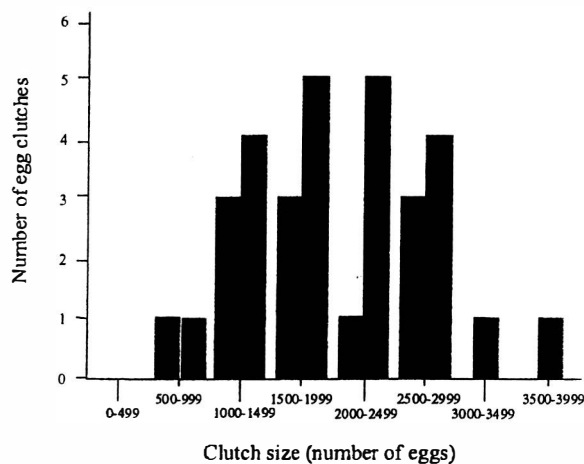Mean female fecundity was 1839 eggs in 1999 (SE=198, n=11), and 2045 eggs in 2000 (SE =170, n

=21). The fecundity, presented in Fig. 1, was not significantly different between years ($t_{(30)}$ = 0.75, P=0.46).

In 2000, we recaptured 17 of the 62 male toads marked in 1999. Male annual survival was therefore at least 0.27. The survey of potential habitat patches in the surroundings of Mellery (>10 km radius) makes emigration unlikely as an explanation for the missing toads. On the other hand, we cannot discriminate between mortality and a possible skipping of breeding activity by some males in the population under study, though the latter behaviour is not common in the species. The estimate of male survival is, therefore, minimal.

Skeletochronology showed that a sample of 34 male toads were between 2 and 4 years old in 1999 (Fig. 2). No relationship was found between age and morphological variables (mass: Kendall's τ=0.125, n=34, P=0.36; length: Kendall's τ=0.041, n=34, P=0.76). Growth rate therefore varied between individuals.

Mean length of males did not vary significantly between the two seasons (mean length in 1999 = 58.2 mm, SE=0.7, n=62; mean length in 2000 = 58.8 mm, SE=0.5, n=84; $t_{(144)}$=0.72, P=0.47). However, mean mass of males was greater in 1999 (mean mass = 18.2 g, SE=0.6, n=62) than in 2000 (mean mass = 16.2 g, SE=0.6, n=93), and this difference was significant (Kruskal-Wallis χ^2 approximation = 4.89, df=1, P=0.03). Consequently, the condition of toads, as assessed by the mass-to-length ratio for the 55 to 60 mm size-class (in order to limit the effect of a possible non-linear relationship between mass and length) was significantly lower in 2000 when compared with 1999 (mean ratio in 1999 = 0.29 g/mm, SE=0.009, n=21; mean ratio in 2000 = 0.26 g/mm, SE=0.005, n=30; Kruskal-Wallis χ^2 approximation = 12.25, df=1, P< 0.001). In 2000, the mean mass

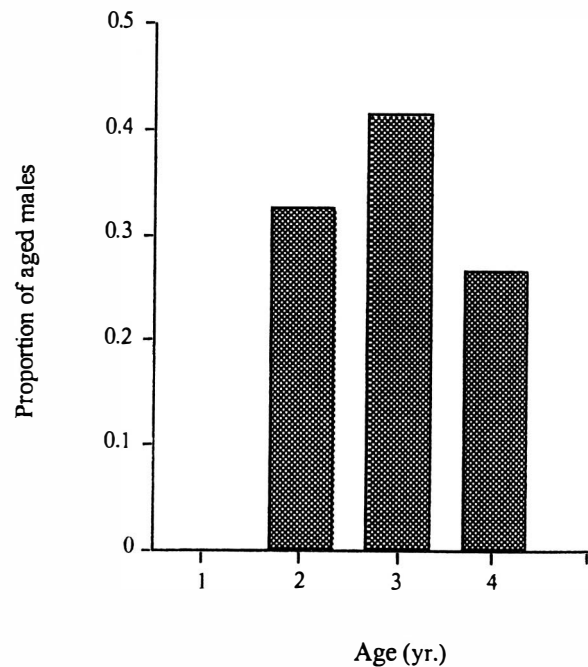FIG 1. Clutch size of *Bufo calamita* at Mellery. Grey bar 1999, black bar 2000.

FIG 2. Age structure of the male breeding population of *Bufo calamita* at Mellery in 1999, expressed as the proportion of the total number of analysed males (n=34).

TABLE 1. Difference in mass and length (in 2000) for male *Bufo calamita* present or not present in the choruses of Mellery in 1999.

	Present in 1999	Mean	SE	n	Statistic $_{(df)}$ and value	P
Mass (g)	Yes	19.2	0.9	17	Kruskal-Wallis $\chi^2_{(1)}$ approximation=10.63	<0.01
	No	15.5	0.4	76		
Length (mm)	Yes	63.0	0.9	17	$t_{(82)}$=4.48	<0.001
	No	57.8	0.5	67		

and length of toads that had been marked in 1999 were significantly greater than those of toads not observed in choruses in 1999 (Table 1).

REPRODUCTIVE ACTIVITY

Males. Breeding activity in the toad population lasted for 60 days in 1999 and 48 days in 2000. The sizes of the three choruses (one in 1999, two in 2000) fluctuated during the breeding season and reached a maximal value in each site around the mid-season (12 May in 1999, 13 May in 2000). As shown in Fig. 3, arrival dates of males in the choruses varied strongly between individuals: unmarked toads were caught each time we visited the two

choruses. The effect of the morphological variables (mass and length) was tested against the arrival date. Table 2 summarizes the results of the regression analyses. Arrival date was related to length in 1999, and to length and mass in 2000. As shown in Table 2, longer males tended to join the chorus earlier in both years, whereas body mass tended to be lower among males joining the chorus later in 2000.

Chorus composition varied during both seasons: some males stayed in the chorus for the entire season, while others left after a few nights. On average, males stayed in the chorus for 33% of the season (SE =4, range 2-92%, n=62) in 1999 (permanent pond only) and for

TABLE 2. Relationship between body size (mass and length) and arrival date of male *Bufo calamita* in the chorus: summary of multiple regression analyses.

Year	Variable entered in the model (units)	Parameter estimate	SE	F for model	df$_{(model,error)}$	t-value	P
1999	MASS & LENGTH			3.92	2,50		0.02
	Intercept	95.68	33.33			2.87	0.006
	Mass (g)	0.86	0.92			0.93	0.35
	Length (mm)	-1.58	0.82			1.92	0.006
2000	MASS & LENGTH			16.81	2,81		<0.001
	Intercept	123.81	21.11			5.86	<0.001
	Mass (g)	1.36	0.61			2.21	0.03
	Length (mm)	-2.22	0.49			4.44	<0.001

TABLE 3 Summary of multiple regression analyses for chorus attendance by *Bufo calamita* at Mellery. Three explanatory variables (mass, length and arrival date) were included to predict the proportion of the total duration of the chorus for which each male was present.

| Year	Variable entered in the model (units)	Parameter estimate	SE	F for model	df$_{(model,error)}$	t-value	P
1999	MASS, LENGTH & ARRIVAL DATE			9.89	3,49		<0.001
	Intercept	-1.56	0.64			-2.42	0.02
	Mass (g)	-0.05	0.02			-2.98	0.004
	Length (mm)	0.05	0.01			3.35	0.002
	Arrival date (days)	-0.01	0.00			-3.07	0.003
2000	MASS, LENGTH & ARRIVAL DATE			38.87	3,80		<0.001
	Intercept	-0.28	0.49			-0.58	0.56
	Mass (g)	-0.03	0.01			-2.41	0.02
	Length (mm)	0.02	0.01			2.26	0.03
	Arrival date (days)	-0.02	0.00			-8.13	<0.001
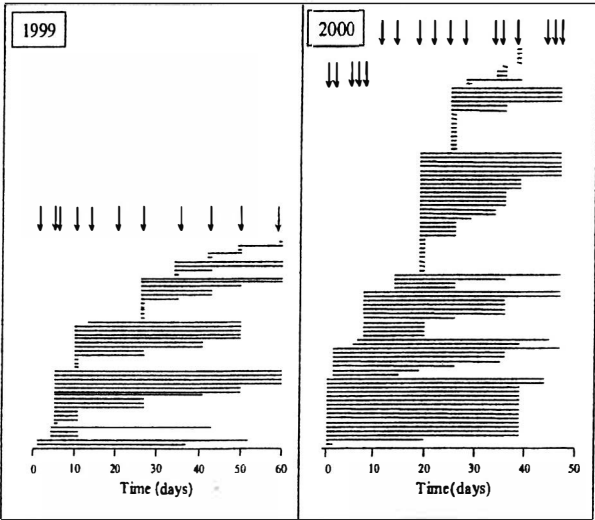
FIG. 3. Arrival date and minimum residence time of male *Bufo calamita* at Mellery, at the Lake site in 1999, and at Lake and Pond sites in 2000. Each line represents one male, and day 0 corresponds to the beginning of male calls at Mellery each year. The arrows indicate the nights of censuses.

FIG. 4. Calling activity of male *Bufo calamita* at Mellery in 2000. Calling activity, the proportion of observations of calling *vs* silent behaviour, was assessed for males observed at least three times in a chorus.

38% of the season in 2000 (SE=3, range 2-94%, n=92). We found no significant between-year difference in length of chorus attendance (Kruskal-Wallis χ^2 approximation = 2.03, df=1, P=0.15). We tested the effect of three variables (body mass, length and arrival date) on male chorus attendance. The arrival date was a significant determinant of chorus attendance in both years: toads that joined the chorus first showed a longer chorus attendance (Table 3). Chorus attendance was also related to the individual's length and body mass (Table 3).

Calling activity was assessed in 2000 only. Males that were observed at least three times in a chorus called in 53% of observations, on average (Fig. 4). Calling activity of males was related to length and body mass (Table 4), but neither to arrival date nor to length of chorus attendance. Longer and heavier males exhibited significantly greater calling activity than smaller males (Table 4). Toads present in the chorus in 1999 called for 69% (SE=6, n=11) of the time they spent in the choruses in 2000, whereas toads not encountered in 1999 called
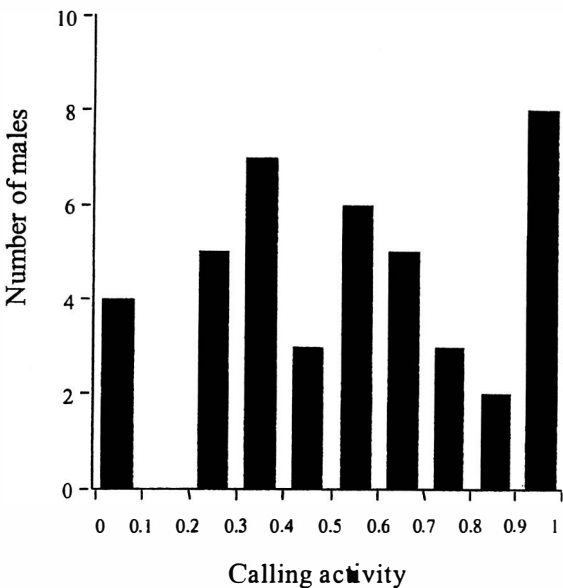
for only 48% (SE=6, n=32) of the time in 2000 (Table 4). The latter difference may have been due to the morphological attributes of the toads, rather than to their experience (Table 4), as the two groups differed in body size in 2000 (Table 1).

Females. In 1999, 22 females spawned between the 1st and the 48th nights and no clutches were found during the last 12 nights of the chorusing season. For those clutches that were counted, we found no significant relationship between laying date and clutch size (Kendall's τ=0.33, P=0.24, n=11). In 2000, 51 females spawned between the 6th and the 33rd nights in the permanent pond and between the 3rd and 45th nights in the ephemeral pond; calling activity of males lasted for 48 nights that year. We counted the eggs of 21 clutches and, unlike in 1999, found a significant tendency for clutch size to be smaller later in the season (Kendall's τ= 0.40, P=0.01).

We were unable to relate clutch size to the body size of individual females. Nevertheless, we used published relationships between fecundity and length of females in

TABLE 4. Effects of morphological attributes, behaviour and previous history of male *Bufo calamita* (presence, arrival date and chorus attendance in 1999) on calling activity in 2000.

Tested variable	Statistic	τ	χ^2	P	n
Length	Kendall's correlation	0.34		<0.01	42
Mass	Kendall's correlation	0.31		<0.01	43
Arrival date in 2000	Kendall's correlation	-0.09		0.45	43
Chorus attendance in 2000	Kendall's correlation	0.09		0.44	43
Arrival date in 1999	Kendall's correlation	-0.07		0.79	11
Chorus attendance in 1999	Kendall's correlation	0.04		0.87	11
Presence in 1999	Kruskal-Wallis test		4.55	0.03*	Present=11 Absent=32

TABLE 5. Natterjack toad clutch size recorded within the distribution of the species [1]This study; [2]Beebee (1979); [3]Banks & Beebee (1986); [4]Banks & Beebee (1988); [5]Tejedo (1992b); [6]Tejedo (1992c); [7]Kadel (1975).

Country	Range of clutch size
Belgium	700-3900[1]
Britain	2700-7200[2,3,4]
Germany	3000-7000[2]
Spain	3000-7000[2,5,6]
Sweden	3200-4000[2]
Switzerland	3600[7]

two populations in Britain (Banks & Beebee, 1986), one in Germany (Hemmer & Kadel, 1975) and one in Spain (Tejedo, 1992c) to investigate whether the relatively low fecundity we observed (Table 5) was due to the small size of the females. Predicted fecundities for females between 48 mm and 71 mm SVL (the extreme sizes at Mellery in 1999) were calculated from these four relationships. The relationship from the Spanish population predicted correctly the fecundity at Mellery (ranging from 920 to 2729 eggs per clutch in 1999), whereas the British and German relationships did not (Table 6).

TABLE 6. Expected clutch size for females ranging from 48 to 71 mm SVL, according to formulae linking clutch size to snout-to-vent length (SVL): [1]Banks & Beebee (1986); [2]Hemmer & Kadel (1971); [3]Tejedo (1992c).

Formulae	Expected clutch size (48-71 mm SVL toad)	Site - Country
$F = 99.2 \times SVL - 3204$	1558 – 3839	Cumbria – Britain[1]
$F = 114.6 \times SVL - 2110$	3391 – 6027	Hampshire – Britain[1]
$F = 158 \times SVL - 4552$	3032 – 6666	Germany[2]
$F = 0.031 \times SVL^{2724}$	1177 – 3421	Sierra Morena – Spain[3]

TABLE 7. Logistic regressions of reproductive success of males *Bufo calamita* on five potentially explanatory variables: mass, length, arrival date, chorus attendance, and calling activity.

	Parameter estimate	SE	Wald χ^2	$P > \chi^2$
Intercept 0	2.97	1.09	7.46	<0.01
Intercept 1	4.18	1.14	13.44	<0.001
Intercept 2	6.49	1.49	18.95	<0.001
Mass	-0.12	0.06	3.40	0.065
Intercept 0	8.00	3.34	5.75	0.016
Intercept 1	9.13	3.37	7.32	<0.01
Intercept 2	11.46	3.52	10.61	<0.01
Length	-0.12	0.05	4.48	0.034
Intercept 0	-0.44	0.39	1.26	0.26
Intercept 1	0.94	0.43	4.85	0.03
Intercept 2	3.35	1.02	10.73	<0.01
Arrival date	0.12	0.03	15.93	<0.001
Intercept 0	2.65	0.55	23.04	<0.001
Intercept 1	4.01	0.65	37.65	<0.001
Intercept 2	6.39	1.15	30.61	<0.001
Chorus attendance	-3.58	0.941	14.53	<0.001
Intercept 0	0.47	0.62	0.60	0.43
Intercept 1	1.59	0.67	5.62	0.02
Intercept 2	3.69	1.14	10.46	<0.01
Calling activity	0.09	1.00	0.01	0.93

REPRODUCTIVE SUCCESS OF MALES

As the number of spawning females was lower than the number of males in the choruses, we tried to identify morphological and behavioural features of successful males. Of the 98 males estimated to be present in 2000, we identified 24 in amplexus. Among the latter, eight reproduced at least twice and one three times. Five variables (body mass, length, arrival date, chorus attendance and calling activity) were used in separate logistic regressions with reproductive success as the dependent variable (summarized in Table 7). This procedure allowed us to detect effects of chorus attendance and arrival date and, to a lesser extent, the male toad's length (Table 7). Mating males were longer, joined the chorus earlier in the season, and stayed in the chorus for a long time.

DISCUSSION

The first result of our study was the very low clutch size of natterjacks at Mellery, compared with other populations (Table 5). In the natterjack, clutch size generally increases with body mass and length (Tejedo, 1992c), so the low fecundity we recorded at Mellery might have been due to the small size of the females. The females we caught were indeed as small as the males of the population (i.e. 48-71 mm in 1999). However, the comparison with other populations (Table 6) suggests that small size alone did not account for the small clutch size. This result is striking, as the ecology of Belgian natterjacks shares common features with those of British and German populations, while Spanish natterjacks differ strongly from northern conspecifics, as follows: (1) Spanish natterjacks are explosive breeders (their breeding season lasts only 2-3 weeks, compared to eight weeks in Belgium); (2) the density of their breeding populations is higher; (3) they are generally larger in size than British and German toads; and (4) their relative fecundity for comparable-sized individuals is known to be smaller (Tejedo, 1988, 1992c). As Spanish natterjacks are larger, clutch sizes as low as those at Mellery were not observed in natural Spanish populations (Tejedo, 1992b). Therefore, the low fecundity observed at Mellery cannot be entirely attributed to the youth of the females. Low fecundity could also be due to the poor condition of the females, resulting from unfavourable environmental conditions (for instance, high local density or low prey availability). We cannot at this time discriminate between the two hypotheses because the quality of the habitat and the density were not known precisely.

The minimum estimate of annual survival rate of 27% obtained in our population was very low when compared with values of 50-80% reported by Sinsch & Seidel (1995), Griffiths (1997) and Stephan et al. (2001). This estimated survival rate is, in fact, a between-year recapture rate. We cannot claim that all toads not recaptured in 2000 were indeed not alive at this time. The high capture probability we recorded during MRR (we marked 95% of the estimated male population every year) excludes a low catchability of individuals at the breeding sites as an explanation for this small value. Some males may not have attended the chorus in 2000, and therefore may not have been available for capture during the MRR study, although in other studies it has been shown that male natterjacks take part in reproductive activity almost every year (Sinsch & Seidel, 1995). Another possible reason for a low recapture rate is dispersal of some individuals between years, but – given the lack of occupied potential natterjack breeding sites within a 10 km radius – this explanation seems unlikely. Moreover, adult natterjacks, particularly males, usually show high breeding site fidelity (Denton & Beebee, 1993a; Tejedo, 1992c). The hypothesis that the between-year recapture rate really reflected a low survival rate is supported by the low body condition we recorded for males. The body condition, estimated by the mass-to-length ratio for the 55-60 mm size-class ($0.26 - 0.29$ g mm^{-1}), is at the lower end of the range of values for seven British populations, where this ratio (for the same size-class) ranged between 0.29 and 0.44 g mm^{-1} (Denton & Beebee, 1993b). As in other species with indeterminate growth (Forsman, 1993; Civantos, Salvador & Veiga, 1999; Gotceitas et al., 1999; Morey & Reznick, 2001), the winter survival of a natterjack is likely to be dependent on its general condition and on its deposition of fat bodies. We could therefore suggest (according to Williams, 1966) that toads in poor condition have a low survival probability, and that the recapture rate we recorded truly reflects a low male survival rate at Mellery. The age structure observed for males in this study site reinforces this hypothesis. Despite the fact that only 52% of the male population was aged by skeletochronology, all size-classes were sampled: only the smallest and the largest we marked were not aged. Moreover, as calling males have been present at Mellery at least since 1995, we can exclude a very recent colonization of the site as an explanation of the youth of the toads.

One of the most central questions in evolutionary biology is the manner in which morphological and behavioural traits affect individual reproductive success. In our population, as in most other natterjack populations, the mean reproductive success of a male was less than one amplexus per year. Some individuals had no access to reproduction, whereas others fathered thousands of potential descendants. So, the question is: who were these reproducers and how did they succeed? As shown in Tables 2 and 3, whereas longer males joined the chorus early in the season and achieved longer chorus attendance than smaller individuals, greater male body mass was associated with late arrival and short chorus attendance. This apparently contradictory result could be interpreted in terms of "body shape" – for a given length, toads with low body mass (i.e. slim toads) show an early arrival date and a long chorus attendance, while big males came later and left the chorus quickly. This difference in reproductive effort could be

due to the general poor condition of the toads at Mellery. As their probability of survival is low, and the possibility of a future reproduction is unlikely, some toads – and particularly the slimmest – might prefer to invest more in reproduction. In the natterjack, as in other anurans, male mating success was usually positively related to the duration of chorus attendance (Halliday & Verrell, 1986; Denton & Beebee, 1993a; Sinsch & Seidel, 1995). Moreover, the calling behaviour was decisive in the reproductive success of male natterjacks: a calling male obtained at least twice as many matings as a silent (or satellite) male (Arak, 1988; Tejedo, 1992a). Chorus attendance and calling, the keys to reproduction, are expensive behaviours (Taigen & Pough, 1985; Tejedo, 1992a; Murphy, 1994). We might expect that toads calling and staying in the chorus for a long time do this in order to mate. However, the pattern of reproductive success we observed at Mellery is unusual – reproductive success was independent of calling activity, and depended more on the male's arrival date and chorus attendance. This could be explained by the irregular arrival date of the females: for both years, twice as many females came to reproduce in the first half of the season as in the second. Therefore, males joining the chorus after the mid-season had less than half the breeding opportunities compared to those males involved at the beginning of breeding activity. The absence of mating gain for calling males could be understood in the light of the satellite tactic. Arak (1988) showed that, in high-density choruses, the reproductive success of calling males was reduced by the presence of satellites in their surroundings. While a small male adopting the satellite tactic increases his probability of mating, calling males are really parasitized by the presence of satellite(s), and therefore see their reproductive success reduced. Aquatic habitat area was small and the satellite tactic was largely used at Mellery, where toads called for a mean of 53% of our records, and never more than 12 callers were recorded in the concrete pond despite the presence of 30 males on some nights. We therefore suggest that satellites gained as many matings as callers and in this way decreased the reproductive success of callers. This may explain why we noticed no difference in reproductive success between caller and silent males (Table 7).

All the demographic features we observed correspond to consequences of excessive density and indicate that the population is under stress. Effective conservation measures therefore imply enlargement of existing suitable terrestrial habitats and management of ponds in the vicinity.

ACKNOWLEDGEMENTS

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A REVISION OF THE *SCAPHIOPHRYNE MARMORATA* COMPLEX OF MARBLED TOADS FROM MADAGASCAR, INCLUDING THE DESCRIPTION OF A NEW SPECIES

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A revision of the available material hitherto assigned to the endemic Malagasy microhylid toad *Scaphiophryne marmorata* finds this taxon to be a complex of three species. In this study we resurrect *Scaphiophryne spinosa* Steindachner, 1882 from the synonymy of *S. marmorata* and describe a new species from central eastern Madagascar. These three *Scaphiophryne* species are characterized by their distinctly expanded terminal finger discs, a character only shared with *S. gottlebei*. *S. spinosa* is characterized by a highly granular back, with large spiny tubercles above the forelimb insertion and in the tympanic region, while *S. marmorata* is dorsally covered by less prominent and more regular tubercles. The new species is distinguished from both *S. marmorata* and *S. spinosa* by its large body size (SVL 47-60 mm), a smoother dorsal skin, and reddish terminal finger discs in life. *S. spinosa* is distributed in low- and mid-altitude rainforests along the east coast, whereas the new species is only known from mid-altitude rainforest in the Fierenana region. In contrast, records of *S. marmorata* include eastern mid-altitude rainforests and several more arid western sites.

Key words: Amphibia, Anura, Madagascar, Microhylidae, frog taxonomy

INTRODUCTION

Several of the anuran genera of Madagascar belong to diverse endemic radiations such as the Mantellidae or the microhylid subfamily Cophylinae (Vences & Glaw, 2001). Other genera, in contrast, are less speciose and have uncertain affinities within their respective higher taxa. Such genera are often distributed in the xeric regions of western Madagascar and show reproductive modes typical for arid environments (Vences *et al.*, 2000*a*).

One of these groups is the genus *Scaphiophryne*, which – together with the monotypic *Paradoxophyla* – is classified in the endemic subfamily Scaphiophryninae (Blommers-Schlösser & Blanc, 1991), or even in the separate family Scaphiophrynidae (Dubois, 1992). *Scaphiophryne* are unique in the morphology of their tadpoles, which are intermediate between the ranoid and the microhylid type (Wassersug 1984). They reproduce in lentic, often temporary waters, and lay a large number of small eggs (Blommers-Schlösser, 1975; Vences *et al.*, 2002*a*). This reproductive mode is typical for seasonal areas such as savannas (e.g. Rödel, 2000), but

several species of *Scaphiophryne* are also known from the mountains and rainforest areas of central and eastern Madagascar. According to Blommers-Schlösser & Blanc (1991) these comprise *Scaphiophryne madagascariensis*, *S. marmorata* and *S. pustulosa*. Three further species – *S. brevis*, *S. calcarata* and *S. gottlebei* – are known from western and southern Madagascar.

Vences *et al.* (2002*b*) provided evidence that the name *Pseudohemisus pustulosus* Angel & Guibé, 1945 is a junior synonym of *Calophrynus madagascariensis* Boulenger, 1882. Consequently, they applied the name *Scaphiophryne madagascariensis* to populations previously assigned to *S. pustulosa*, and verified that no available name exists for specimens from the Andringitra Massif in south-eastern Madagascar which has thus far been referred to as *S. madagascariensis*.

Scaphiophryne madagascariensis and the new form from Andringitra are known from high altitudes along the central mountain chain of Madagascar, and are characterized by lacking greatly expanded terminal discs on fingers and toes. In contrast, *S. marmorata* is known from forests in eastern Madagascar, and from relict forests in the arid west (Glaw & Vences, 1994). It is characterized by its expanded terminal finger discs (and slightly expanded toe discs), a state that so far was thought to be shared by only one further species, *S.*

gottlebei from Isalo in south-western Madagascar (Busse & Böhme, 1992). Recent fieldwork revealed the presence of additional species with this character in eastern Madagascar. We herein revise the material of green-coloured *Scaphiophryne* with expanded finger discs available to us, resurrect the name *Scaphiophryne spinosa* Steindachner, 1882 and describe one highly differentiated new species.

MATERIALS AND METHODS

The present revision is based on material in the following collections: The Natural History Museum, London (formerly British Museum of Natural History) (BMNH); Muséum National d'Histoire Naturelle, Paris (MNHN); Museo Regionale di Scienze Naturali, Torino (MRSN); Naturhistorisches Museum Wien (NMW); University of Michigan, Museum of Zoology (UMMZ); Zoölogisch Museum Amsterdam (ZMA); Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK); Zoologische Staatssammlung München (ZSM).

Specimens were measured with calipers to the nearest 0.1 mm. Measurements taken were SVL (snout-vent length), HW (head width at the maxillary rictus), HL (head length, from the maxillary commissure to the snout tip), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), HAL (hand length, from the carpal-metacarpal articulations to the tip of the longest finger), FORL (forelimb length, from the axil to the tip of the longest finger), HIL (hindlimb length, from the cloaca to the tip of the longest toe), FOL (foot length, from the tarsal-metatarsal articulations to the tip of the longest toe), FOTL (foot length including tarsus, from the tibiotarsal articulation to the tip of the longest toe), IMTL and IMTH (maximum length and height of inner metatarsal tubercle), FD4 (maximum width of the terminal disc of fourth finger). The sex and maturity of preserved specimens were either determined by obvious secondary sexual characters of males in breeding conditions (vocal sac) or by dissection and gonad examination.

Advertisement calls were recorded in the wild or in captivity and were analysed on a PC using the software Cooledit (Syntrillium Corp.). Statistical analyses were carried out using SPSS for Windows, version 10. Measurements are given as range, with mean ± standard deviation in parentheses. DNA sequences of a fragment (ca. 540 base pairs, depending on the number of indels) of the mitochondrial 16S rRNA gene were obtained using primers and protocols given in Vences *et al.* (2000*b*), and have been deposited in Genbank. Voucher specimens for sequences and Genbank accession numbers are as follows: *Scaphiophryne boribory* sp. n. (ZSM 644/2000, AJ314810), *S. marmorata* (ZSM 303/2000, AJ417567), *S. spinosa* (ZSM 1154/2001, AF215383).

RESULTS AND SPECIES ACCOUNTS

During the revision of the available material thus far attributed to *S. marmorata,* we noted the existence of two distinct species: a large species with a very granular and spiny dorsal skin, and a smaller, less granular species. Careful examination showed that the less granular species is distinguished from the spiny species by the following characters: (1) smaller body size (male SVL 32-49 mm vs. 40-48 mm; Table 1); (2) presence of a variable number of regular larger granules on head and body, usually including a symmetrical pair of elongated tubercles in the shoulder region and a smaller pair on the posterior dorsum, these tubercle pairs being especially distinct in subadults (vs. a large number of irregular dorsal tubercles of different size, and absence of elongated symmetrical tubercles in the shoulder region); (3) absence of large spiny tubercles above forelimb insertion, at posterior end of maxilla, and in tympanic region (vs. presence); (4) tympanum often faintly recognizable, its horizontal diameter larger than 50% of eye diameter (vs. tympanum usually totally concealed, smaller than 50% of eye diameter – if visible); (5) ventral pattern variable, but often (especially in juveniles) with contrasting dark-light marbling, the dark colour extending onto the posterior belly and the femur (vs. absence of contrasted marbling, usually dark on throat, chest and anterior belly, light on posterior belly and femur). These differences were already visible in juveniles of both species (e.g. UMMZ 191157; ZSM 303/2000). We also recognized a third larger species, with a smoother dorsal body surface and reddish discs on the fingers and toes. The three species showed a relevant molecular differentiation in the sequenced fragment of the 16S rRNA gene (see comments in the section of *S. boribory* below).

SCAPHIOPHRYNE MARMORATA BOULENGER, 1882 (FIG. 1-2)

Diagnosis. A *Scaphiophryne* with highly expanded terminal discs on the fingers; dorsally green with symmetrical dark markings, and lacking pink markings; adult SVL 32-49 mm; typically two symmetrical pairs of larger tubercles, an elongated pair in the shoulder region and a smaller pair on the posterior dorsum; absence of large spiny tubercles above forelimb insertion, at posterior end of maxilla, and in tympanic region; tympanum typically visible with a horizontal diameter > 50% of eye diameter; ventral pattern often with contrasted dark-light marbling, the dark colour extending onto the posterior belly and the femur.

Material examined. BMNH 1947.2.30.81 (holotype by monotypy; East Betsileo, collected by W. D. Cowan); MNHN 1883.581 (Madagascar); MNHN 1953.241 (large series of juveniles; Andasibe); MNHN 1962.923 (Andasibe, collected by E. R. Brygoo); MNHN 1962.924 (Andasibe, collected by J. Arnoult); MNHN 1965.315 (Andasibe); MNHN 1975.1569 (Andasibe); UMMZ 211504-211505 (Zahamena reserve, Volotsangana river, 850 m; collected by C. J.
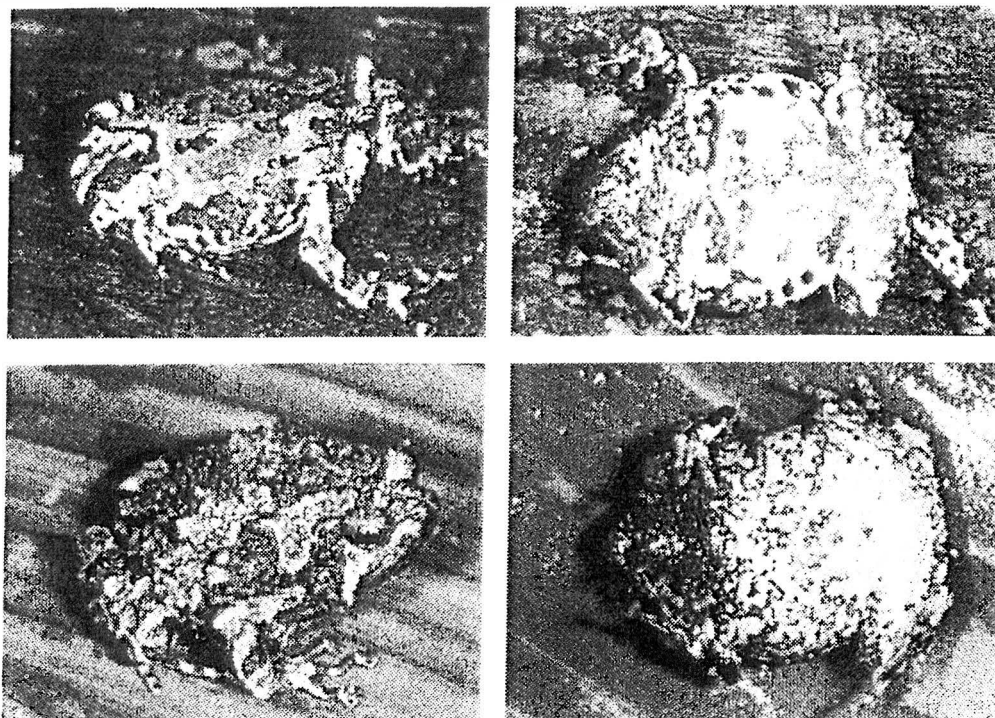
FIG. 1. Comparative dorsolateral and ventral views of *Scaphiophryne marmorata* (upper figures; Andasibe, 1995; specimen not collected) and *S. spinosa* (lower figures; An'Ala, close to Andasibe, 1996; specimen not collected).

Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa and A. Razafimanantsoa on 26 February 1994); UMMZ 219487 (Kirindy forest, 20° 04.099'S, 44° 39.403'E; collected by C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa and A. Razafimanantsoa on 31 January 1996); UMMZ 219488-219489 and 219491-219495 (Antranopasasy, 18° 42.481'S, 44° 42.981'E, Bemaraha reserve; collected by C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana, A. Razafimanantsoa and A. Razafimanantsoa, 8-11 March 1996); UMMZ 225606 and UMMZ 225608-225613 (Ampahanana forest, near Fierenana, 18° 29.53'S, 48° 26.68'E, 1000-1200 m altitude; collected by O. Ramilison, N. Rabibisoa and D. Foley IV on 22-24 January 1998);

FIG. 2. *Scaphiophryne marmorata*, specimen from western Madagascar (Kirindy).

UMMZ 227489 (Isalo region; collected by local collectors); UMMZ 227499 (Namoroka reserve, 16° 28.189'S 45° 20.906'E; collected by C. J. Raxworthy, J. Rafanomesantsoa, J. B. Ramañamanjato, A. Razafimanantsoa and A. Razafimanantsoa on 6 December 1996); ZMA 6877 (1077 and 1121-1122) (Andasibe; collected by R. M. A. Blommers-Schlösser on 24 December 1972); ZFMK 52760 (Andasibe, collected by local collectors in 1991); ZSM 303/2000 (Andasibe, collected by F. Glaw on 10 April 2000).

Distribution. The type locality is the region of East Betsileo. Other precise and reliable localities are the following: (1) Zahamena, (2) Andasibe, (3) Fierenana, (4) Namoroka, (5) Bemaraha, (6) Kirindy, and (7) the Isalo region. The species is distributed in mid-altitude rainforest localities of central eastern Madagascar, and is known from four sites in western Madagascar (Fig. 3). This combined humid and arid habitat distribution is highly unusual for an amphibian species in Madagascar. The localities Foizana, Ampasy and Ambana in Blommers-Schlösser & Blanc (1991) are here assigned to *S. spinosa* (see below).

Natural history. The dissected females UMMZ 227489 and UMMZ 225610 contained a large number of small oocytes (diameter 1.2 mm) which were yellowish with a large dark brown pole. Advertisement calls are unknown.

Comments. The holotype of *S. marmorata* (BMNH 1947.2.30.81) is a subadult specimen (see Table 1 for measurements). However, with the exception of SVL, the *marmorata* type agrees well with the diagnosis given above. Among the specimens examined, some variation in coloration is apparent: specimens from the east are
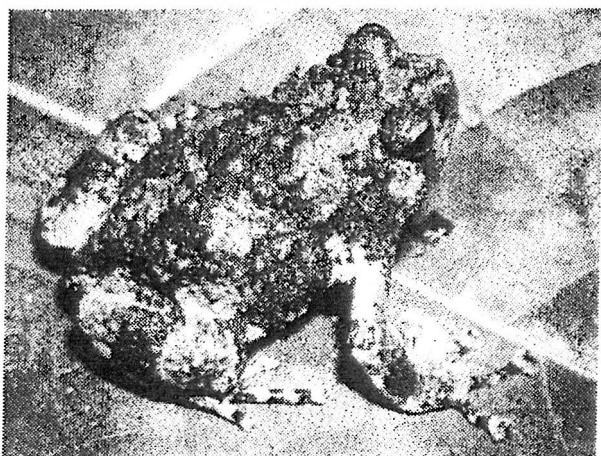
TABLE 1. Morphometric measurements (all in mm) of adult specimens of *Scaphiophryne marmorata*, *S. spinosa* and *S. boribory*, and of the subadult holotype of *S. marmorata*. For abbreviations of measured variables, see Materials and Methods; further abbreviations used: M (male); F (female); SA (subadult); HT (holotype); PT (paratype); RHL (relative hindlimb length: point reached by tibiotarsal articulation when the hindlimb is adpressed along the body). RHL is coded as follows: 0, the tibiotarsal articulation does not reach the forelimb insertion; 1, it reaches the forelimb insertion; 2, it reaches between forelimb and tympanic region; 3, it reaches tympanic region.

Specimen	Sex	Status	SVL	HW	HL	TD	ED	END	NSD	NND	HAL	FORL	HIL	FOTL	FOL	IMTL	IMTW	FD4	RHL
S. marmorata																			
BMNH 1947.2.30.81	SA	HT	29.1	10.0	8.9	(1.8)	3.0	1.7	1.6	2.5	10.0	20.5	41.8	20.6	14.3	2.9	2.0	—	2
UMMZ 219487	M	—	41.6	13.9	11.8	(2.3)	3.5	2.9	1.1	2.9	14.8	31.2	56.9	26.3	18.5	3.7	2.6	2.2	1
UMMZ 219489	M	—	39.9	13.2	11.0	—	3.2	2.3	1.5	2.2	14.1	28.3	51.4	25.1	17.9	2.9	2.3	2.5	1
UMMZ 225606	M	—	35.8	13.1	10.5	(1.8)	3.5	2.3	1.3	2.8	12.4	25.5	49.0	23.5	17.0	2.6	2.3	2.0	1
UMMZ 225608	M	—	32.4	11.5	9.6	—	3.5	2.0	1.3	2.2	11.4	21.8	45.1	20.7	15.4	3.2	2.3	1.6	1
UMMZ 225612	M	—	33.0	11.7	8.8	—	3.1	2.0	1.4	2.4	11.7	23.9	43.3	21.6	15.2	2.7	2.3	1.9	1
ZMA 6877 (1077)	M	—	35.9	11.8	9.9	—	3.8	2.5	1.4	2.6	12.6	25.5	50.0	23.7	17.4	3.4	2.4	2.2	1
ZMA 6877 (1121)	M	—	35.6	13.3	10.8	(2.0)	4.0	2.2	1.6	2.7	12.8	26.6	46.4	23.3	16.5	3.3	2.2	2.1	1
ZMA 6877 (1122)	M	—	36.2	12.0	10.8	(2.0)	3.7	2.0	1.2	3.0	13.2	26.4	51.1	24.1	17.6	3.1	2.4	2.0	2
UMMZ 211504	F	—	37.5	12.7	11.0	—	3.5	2.4	1.5	3.0	11.6	26.0	49.1	24.2	16.7	3.0	2.3	2.0	1
UMMZ 211505	F	—	43.5	13.3	11.3	(2.0)	3.7	2.5	1.5	2.8	14.3	28.2	57.0	27.4	19.7	3.3	2.4	2.0	1
UMMZ 225609	F	—	34.9	12.2	10.4	—	2.7	2.2	1.3	2.5	12.2	24.6	50.3	23.8	17.0	3.3	2.5	2.1	1
UMMZ 225610	F	—	40.4	12.6	10.6	—	3.0	2.6	1.1	2.9	13.8	27.5	56.0	26.9	19.1	3.3	2.5	2.4	1
UMMZ 225611	F	—	36.6	13.5	11.1	—	3.8	2.3	1.3	3.3	14.0	27.3	54.2	26.1	18.9	3.2	2.8	2.2	3
UMMZ 225613	F	—	39.9	13.9	11.1	—	3.6	2.7	1.2	3.2	13.2	26.7	51.1	25.5	17.9	3.2	2.6	2.1	1
UMMZ 227489	F	—	48.5	17.1	13.6	(2.5)	4.3	3.0	1.8	3.5	(13.0)	30.9	59.5	28.8	20.4	3.9	2.8	3.4	1
S. spinosa																			
UMMZ 191154	M	—	40.4	13.7	12.0	—	3.6	2.7	1.5	2.8	13.9	27.9	58.0	27.6	19.9	3.6	2.5	2.2	2
UMMZ 191155	M	—	39.7	12.5	11.4	—	3.6	2.4	1.9	2.7	13.3	26.4	55.1	26.2	19.0	3.4	2.5	2.3	1
UMMZ 191158	M	—	42.1	14.1	12.3	—	3.7	3.0	1.9	2.5	13.8	31.1	57.5	28.8	20.3	3.0	2.3	2.4	3
UMMZ 198870	M	—	48.3	16.0	13.3	—	4.6	3.4	1.7	3.1	16.5	34.0	66.0	32.3	22.6	3.3	3.0	2.6	2
UMMZ 198871	M	—	46.5	15.8	14.1	(1.6)	4.1	3.1	2.0	3.0	16.4	35.1	65.3	31.3	22.3	4.0	3.0	2.6	3
UMMZ 198872	M	—	48.0	14.9	13.3	(1.7)	4.4	3.3	1.9	3.1	15.7	34.0	66.7	31.3	22.4	3.8	2.8	2.5	1
UMMZ 198873	M	—	46.9	15.8	13.8	(1.4)	4.9	3.0	1.8	3.2	17.2	34.4	64.9	31.5	22.6	3.8	2.7	3.0	2
UMMZ 198874	M	—	45.0	14.3	12.4	—	3.7	2.9	1.7	3.1	16.5	32.4	62.0	31.0	22.1	3.6	2.7	2.5	3
UMMZ 198875	M	—	45.4	14.8	12.2	—	4.1	3.1	1.7	3.0	16.6	32.7	65.3	31.0	22.3	3.6	3.0	3.0	3

|																			
UMMZ 211487	M	—	45.3	14.3	12.6	—	4.6	3.1	1.7	2.5	16.0	31.9	64.0	28.9	20.1	3.2	2.8	2.3	3
UMMZ 211489	M	—	45.7	14.5	12.3		4.1	3.3	1.7	2.6	16.1	30.7	64.5	29.4	21.4	3.3	2.6	2.7	2
UMMZ 211490	M	—	44.7	14.4	12.6	(1.9)	4.7	2.8	1.9	2.9	15.5	31.4	64.3	29.9	21.5	3.6	2.5	2.7	2
UMMZ 211491	M	—	44.3	14.6	12.0	—	4.6	2.9	1.6	3.0	16.3	31.5	64.0	30.6	22.7	3.0	2.7	2.5	3
UMMZ 211492	M	—	46.0	14.8	12.4	—	4.8	2.9	1.6	2.7	16.5	31.5	61.2	31.0	22.6	3.4	2.9	2.6	2
UMMZ 227498	M	—	41.0	13.4	11.8	—	3.3	2.7	1.5	2.8	13.8	30.1	57.3	26.1	19.1	3.5	2.5	2.3	2
MNHN 1973.575	F	—	43.0	14.2	12.1	—	4.4	3.0	1.6	3.0	14.6	31.1	58.7	28.3	20.1	2.9	2.2	2.4	2
NMW 3919	F	HT	43.6	14.5	12.8	—	4.5	2.9	1.8	3.5	14.7	31.1	61.3	28.0	19.6	3.3	2.5	2.4	1
UMMZ 191156	F	—	47.2	14.0	13.0	—	4.0	2.8	1.6	3.3	15.9	32.6	63.6	30.9	21.9	3.7	2.6	2.2	2
UMMZ 211493	F	—	48.2	17.0	13.5		4.5	2.9	1.7	3.4	17.0	34.3	68.9	32.8	24.4	3.6	3.0	2.7	3
S. boribory																			
UMMZ 225614	M	HT	56.3	17.9	14.1	—	4.6	3.2	2.0	3.7	19.4	36.8	71.9	36.2	25.0	3.8	3.0	3.2	0
UMMZ 225615	M	PT	57.4	17.6	13.7	—	4.8	3.0	2.2	4.0	20.5	36.6	76.1	38.6	27.5	4.5	3.5	3.4	0
UMMZ 225617	M	PT	53.3	17.2	13.2	—	4.5	3.6	1.9	2.2	20.1	36.1	74.7	38.2	26.6	3.7	3.2	2.7	1
UMMZ 225618	M	PT	50.8	16.7	13.9	—	5.0	3.2	2.2	3.8	19.5	37.2	75.0	37.0	26.4	4.5	3.4	3.0	1
UMMZ 227490	M	PT	55.2	17.0	13.1	—	4.5	3.1	2.2	3.4	20.5	36.9	74.6	37.9	27.8	4.0	3.1	2.8	1
UMMZ 227492	M	PT	57.0	16.7	13.0	—	4.7	3.3	2.1	3.6	19.2	34.6	74.7	37.9	27.0	4.6	3.2	2.6	1
UMMZ 227494	M	PT	59.1	17.9	13.8	—	4.5	3.2	2.1	3.6	20.1	36.4	80.8	40.8	28.6	4.7	3.4	3.1	1
UMMZ 227495	M	PT	59.8	18.8	14.5	—	5.3	3.2	1.8	3.2	20.0	37.9	77.4	37.8	27.0	4.6	3.5	2.7	1
UMMZ 227496	M	PT	58.5	18.6	14.7	—	4.7	3.3	2.0	3.6	20.8	36.9	78.1	38.7	27.6	4.1	3.6	3.2	1
UMMZ 227497	M	PT	57.5	17.0	13.3	—	4.6	3.1	1.7	3.3	19.4	36.4	80.9	37.6	26.6	4.3	3.0	2.8	1
ZSM 644/2000	M	PT	49.0	16.0	13.3	—	4.2	2.9	1.7	3.1	18.7	37.2	68.4	33.4	24.2	4.0	3.1	2.6	2
ZSM 8/2000	M	PT	54.2	17.7	13.6	—	4.3	3.6	1.8	3.5	20.0	35.8	74.8	36.5	25.8	4.7	3.4	2.7	1
UMMZ 225616	F	PT	56.6	17.4	13.8	—	4.8	3.0	2.0	3.4	19.9	34.6	74.3	37.0	26.3	4.2	3.3	3.4	0
UMMZ 227491	F	PT	59.4	18.2	15.0	—	5.0	3.3	2.0	3.4	20.1	39.4	79.7	39.2	27.1	4.6	3.7	3.2	1
UMMZ 227493	F	PT	55.3	15.9	13.3	—	4.0	3.1	2.0	3.9	19.3	35.7	73.7	35.8	25.9	3.9	3.3	3.1	0
ZSM 7/2000	F	PT	55.8	17.2	13.8	—	4.4	3.5	1.6	3.3	19.9	42.7	79.5	39.0	26.5	3.7	3.1	2.2	2
ZFMK 76102	F	PT	52.6	17.6	14.0	—	5.0	3.5	1.9	3.4	20.7	35.3	76.8	38.1	26.1	4.4	3.0	2.6	1
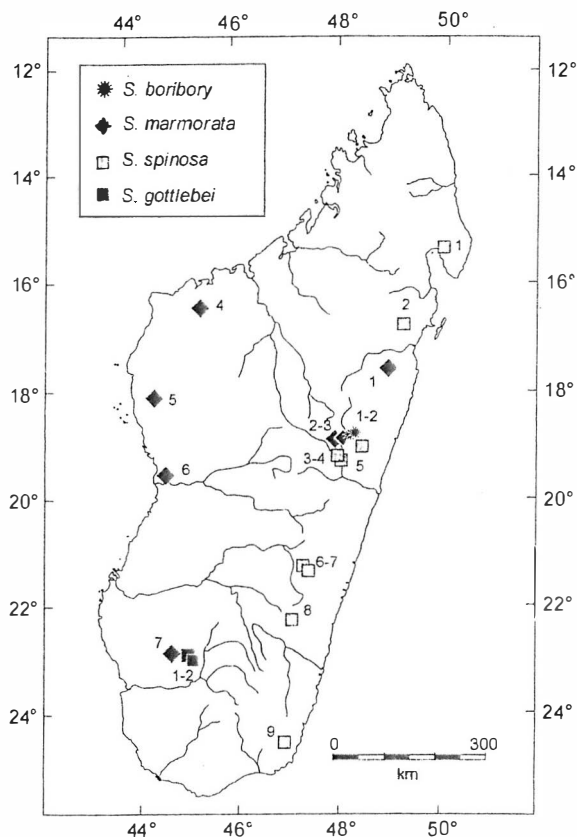
FIG. 3. Distribution map of species of *Scaphiophryne* with expanded finger discs (*S. boribory, S. gottlebei, S. marmorata, S. spinosa*). The only known localities for *S. gottlebei* are in the Isalo region: (1) Vallée (or Canyon) des Singes within the Isalo Reserve (Busse & Böhme, 1992; and Raxworthy, unpublished); and (2) 2 km from Ilakaka (28° 45'29"S, 45° 15'17"E) according to a personal observation by K. Schmidt in 1999. Localities of the other three species are listed in the respective species accounts; locality numbers in the map correspond to those in the text.

usually green, while those from the west often have a largely brown pattern. This variation in coloration and the unusual distribution of this species may suggest that two or more taxa are involved. However, an assessment of the genetic differentiation is necessary before the taxonomic status of the western populations can be reliably determined.

SCAPHIOPHRYNE SPINOSA STEINDACHNER, 1882 (FIG. 1, 4)

Diagnosis. A *Scaphiophryne* with highly expanded terminal discs on the fingers; dorsally green with symmetrical dark markings, and lacking pink markings; adult SVL 40-48 mm; presence of a large number of irregular dorsal tubercles of different size, and absence of elongated symmetrical tubercles in the shoulder region; presence of large spiny tubercles above forelimb insertion, at posterior end of maxilla, and in tympanic region; tympanum typically not visible, or if visible, with a horizontal diameter < 50% of eye diameter; ventral pattern usually lacking contrasting marbling (especially on the belly), and typically dark on throat, chest and anterior belly, light on posterior belly and femur.

Material examined. MNHN 1953.243 (2 juveniles; Moramanga); MNHN 1975.1567 (Ampasy forest, Ivohibe); MNHN 1973.575 (Ambana, Chaînes Anosyennes; collected by C. P. Blanc on 3 December 1971); MRSN A2018 and A2019 (Andasibe region; collected by local collectors in 2001); NMW 3919 (holotype by monotypy; collected at Foizona according to original description, changed to Foizana by Blommers-Schlösser & Blanc (1991) and probably referring to Fizoana near Maroantsetra); UMMZ 191154-191158 (Ambatovaky, 16° 51'S, 49° 08'E, 600-650 m elevation; collected by C. J. Raxworthy, J. B. Ramanamanjato, A. Raselimanana on 8-19 February 1990); UMMZ 198870-198875 (Vatoharanana, Ranomafana National Park, 21° 17'E, 47° 25.5'E, 950 m elevation; collected by C. J. Raxworthy on 31 January 1991); UMMZ 211487 and 211489-211493 (Sahavatoy River, Andringitra National Park, 22° 13'44"S, 47° 01'29"E, 820 m elevation; collected by C. J. Raxworthy, A. Razafimanantsoa and N. Rabibisoa on 26-27 November 1993); UMMZ 227498 (Andasibe region; collected by local collectors). ZFMK 62217 (An'Ala; collected by F. Glaw on 3 February 1996); ZSM 1154/2001 (locality unknown; obtained through the pet trade in 1999).

Distribution. Reliable localities are the following: (1) Foizona (type locality), (2) Ambatovaky, (3) Moramanga; (4) Ankeniheny (photographic record in Glaw & Vences 1994), (5) An'Ala, (6) Ambatolahy (call recordings and photographs of F. Andreone), (7) Vatoharanana (Ranomafana), (8) Ampasy, (9) Ambana, and (10) Andringitra. Except for the type locality, which seems to be at low altitude, the species is known from mid-altitude localities along the Malagasy east coast (Fig. 3).

Natural history. Dissected females (e.g., UMMZ 211493 and 191156) contained a large number of yellowish oocytes with a large dark brown pole of 1.4-1.7 mm diameter. Males at Ranomafana were found calling during the day, in a flooded marsh in rainforest between 09.00 and 17.00 hr (Andreone, pers. com.). Males at Andringitra were heard calling at night, at 20.00 hr, in a small (1 x 3 m) tempory pool of water in rainforest.
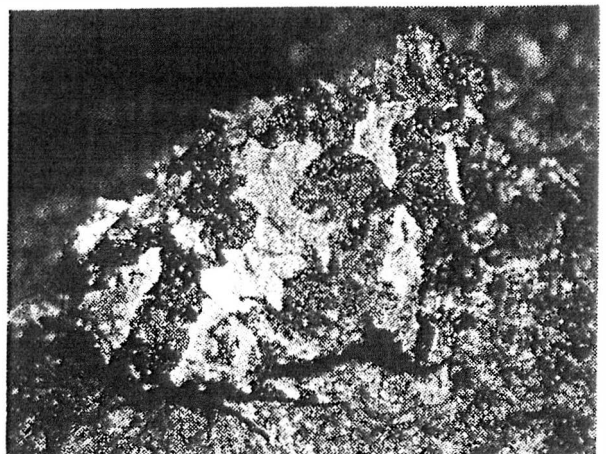

FIG. 4. Adult specimen of *Scaphiophryne spinosa* from Vatoharanana (Ranomafana).

Advertisement call. Recordings of the specimen ZSM 1154/2001 were taken by K. Schmidt in captivity at unknown temperature (about 25°C). The call was a long series of short harmonious notes (Fig. 5). Call duration was 3539-9117 ms (7214±3183 ms, *n*=3). Calls contained 83-200 notes (157±64, *n*=3). Note duration was 25-34 ms (28.6±2.9 ms, n=1 0), and duration of intervals between notes was 9-18 ms (12.3±3.2 ms, *n*=10). Intensity of notes increased from the first to the last notes of the call, the first notes had especially very low intensities. Note repetition rate was 21-23/s. Frequency was 600-1000 Hz.

These calls were similar to those recorded by F. Andreone at Ranomafana (next to Ambatolahy village) on 14 December 1991, ca. 21.00 hr, at 19-20°C air temperature. At least 5-10 specimens called in a chorus in the water, and identification of single calls and notes was therefore very difficult on the sonagrams (not shown). Frequency was 600-1050 Hz, note duration was 21-35 ms, duration of intervals between notes was about 10 ms.

Comments. According to several authors (e. g. Guibé, 1978; Blommers-Schlösser & Blanc, 1991) the holotype of *Scaphiophryne spinosa* is deposited in the NMW, but the specimen has not been so far identified (see Häupl *et al.* 1994, Tiedemann & Grillitsch 1999). However, the original description (Steindachner 1882) is relatively detailed and complete. Some relevant parts of the description are quoted in the following: "*Haftscheiben an den Fingern stark entwickelt, am vorderen Ende breit und in der Mitte des Vorderrandes seicht eingebuchtet. ... Zahllose kleine, mehr oder minder dornenähnliche Wärzchen an der Rückenseite des Körpers, zwischen denen insbesondere an der Oberseite des Kopfes, in der Ohrgegend und an den Seiten des Rumpfes bedeutend grössere konische Wärzchen zerstreut liegen. ... Ein Exemplar, c. 4 1/2 Cm. lang, von Foizona auf Madagascar. ... Kopflänge 14 Mm. Kopfbreite nahezu 20 Mm. Länge der vorderen Extremitäten c. 32 Mm., der hinteren Extremitäten 59 Mm.*" This information, in combination with the excellent drawings of the holotype given in the original description, leave no doubts about the correct assignation of the name *Scaphiophryne*
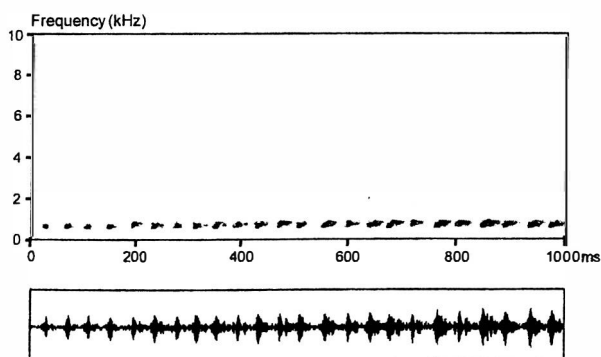

FIG. 5. Sonagram and oscillogram of part of an advertisement call (start of call with first 24 notes) of *Scaphiophryne spinosa* (ZSM 1154/2001), recorded in captivity.

spinosa to the spiny species previously attributed to *S. marmorata* (see above). We also consider that the female NMW 3919 agrees very well with this description and therefore very probably is the holotype of *Scaphiophryne spinosa*, although it bears no label indicating its type status. The colour of NMW 3919 has largely faded to a uniform beige; however, its general appearance agrees with the drawing in Steindachner (1882), and most of its measurements (Table 1) agree with the values given in the original description as quoted above. Differences in head width and hindlimb length values are probably explained by different landmarks of measurements: head width at the level of the maxillary rictus measured herein, maximum head width probably measured by Steindachner (1882); hindlimb length of totally outstretched limbs to the tip of longest toe measured herein, possibly not to the longest toe or not totally outstretched limbs measured by Steindachner (1882). Boulenger (1883) considered *S. spinosa* a junior synonym of *S. marmorata* without stating specific justifications, but he appears to have been influenced by the deposition of an additional adult specimen at the British Museum (the *S. marmorata* holotype is a juvenile).

SCAPHIOPHRYNE BORIBORY SP. N. (FIGS. 6-7)

Diagnosis. A *Scaphiophryne* with highly expanded terminal discs on the fingers that are reddish in life; dorsally green with symmetrical dark markings, and lacking pink markings; adult SVL 47-60 mm; total absence of dorsal tubercles on body in preservation; absence of large spiny tubercles above forelimb insertion, at posterior end of maxilla, and in tympanic region; tympanum not visible; ventral pattern black with white spots.

Distinguished from *Scaphiophryne brevis*, *S. calcarata* and *S. madagascariensis* by the highly expanded terminal discs on the fingers (vs. absent, or only slightly expanded); from *S. gottlebei* by a much larger size (SVL 47-60 vs. 36 mm) and a different coloration (dorsally green with symmetrical dark markings vs. white with green, black and pink markings); from *S. spinosa* by larger adult size (SVL 47-60 mm vs. 40-48 mm), total absence of dorsal tubercles on body in preservation (vs. presence of tubercles), and ventral colour (black with white spotted pattern vs. irregular dark on throat, chest and anterior belly and light on posterior belly and femur); and from *S. marmorata* by larger adult size (SVL 47-60 mm vs. 32-49 mm) and total absence of dorsal tubercles in preservation (vs. presence of at least some large regular tubercles in the head and shoulder region). In life, *S. boribory* is further recognizable by the reddish discs on fingers and toes (not reddish in the other species).

Holotype. UMMZ 225614, adult male, from Ampahanana forest, near Fierenana, 18° 29.53'S, 48° 26.68'E, 1000-1200 m altitude; collected by O. Ramilison, N. Rabibisoa and D. Foley IV on 25 January 1998.

FIG. 6. Adult male specimen of *Scaphiophryne boribory* (ZSM 153/2002) in dorsolateral view.

FIG. 7. Adult male specimen of *Scaphiophryne boribory* (ZSM 153/2002) in ventral view.

Paratypes. UMMZ 225615-225616 (one male and one female, same collecting dates as holotype); UMMZ 225617-225618 (two males, Sahanomanana, near Fierenana river, 18° 29.82'S, 48° 28.21'E; collected by O. Ramilison, N. Rabibisoa and D. Foley IV on 27 January 1998); UMMZ 227490-227497 (six males and two females, Andasibe region; collected by local collectors, December 1996); ZSM 7/2000-8/2000, 644/2000-645/2000, MRSN A2016 and A2017, and ZFMK 76102 (two males, three females and two unsexed adult specimens, Fierenana region; collected by local collectors in 2000). ZSM 153/2002 (adult male, Fierenana region, collected by local collectors in January 2002).

Description of the holotype. Specimen in good state of preservation. For measurements, see Table 1. Body stout; head wider than long, less wide than body; snout rounded in dorsal view, truncate in lateral view; nostrils directed dorsolaterally, not protuberant, nearer to tip of snout than to eye; canthus rostralis indistinct; loreal region flat; tympanum not visible; supratympanic fold rudimentary; tongue ovoid and broad, posteriorly free and not bifid; maxillary teeth rudimentary; vomerine teeth absent; choanae ovoid. Arms moderately slender, faintly marked single subarticular tubercles; indistinct outer and distinct inner metacarpal tubercle; fingers without webbing; relative length of fingers 1<2<4<3, fourth finger clearly longer than second finger; finger discs greatly expanded; nuptial pads absent. Hindlimbs short and rather stout; tibiotarsal articulation does not reach forelimb insertion when hindlimb is adpressed along body; lateral metatarsalia strongly connected; large and sharp inner metatarsal tubercle present, outer metatarsal tubercle not recognizable; a further distinct tubercle present on tarsus (tarsal tubercle); traces of webbing between toes; relative length of toes 1<2<5<3<4; third toe distinctly longer than fifth toe; terminal discs of toes only slightly expanded.

Skin on dorsal surface smooth, with only a few small tubercles on head. Ventral skin smooth except on throat (vocal sac), which appears to be slightly granular. The skin gives an impression that it is oversized, giving a loose and baggy fit on the body.
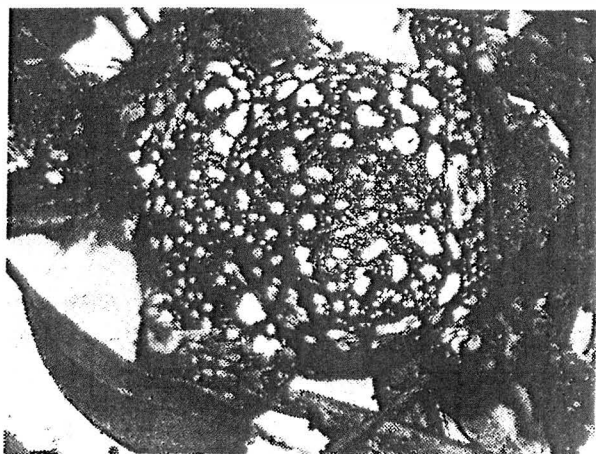
In preservative, dorsally bluish grey with symmetrical, sharply delimited dark brown to black markings. Ventral side dark brown with small, sharply delimited creamy-white spots and vermiculations. Throat blackish. Tips of fingers and toes beige ventrally and beige with dark markings dorsally.

Variation. The available material is very homogeneous. Size is similar between males (SVL 49.0-59.8 mm; 55.7±3.3 mm) and females (47.4-59.4 mm; 54.5±4.1 mm) (Table 1). Males are characterized by a vocal sac which appears to be highly distensible, and which is blackish in specimens from Fierenana (probably captured in breeding condition). In life, some large tubercles were visible on the head and shoulders, but these are not recognizable in preserved specimens which all have smooth dorsal skin. The tympanum is totally concealed in all specimens.

Colour in life. The dorsal colour was a vivid green. The dorsal markings were centrally brown and are surrounded by black. Fingers and toes were largely brown, and their terminal discs were light brown to reddish. Ventrally, black with white spots on the belly; dark brown with green spots on the chest and with three more or less continuous greenish longitudinal bands on the throat. The fingers and toes were ventrally black to dark brown, while their terminal discs were distinctly reddish.

Etymology. The specific name is an unlatinized epithet derived from the Malagasy adjective boribory (= rounded), and refers to the stout and rounded body shape of this species.

Natural history. Dissected females (e.g. UMMZ 227493) contained a large number of light brown oocytes with a dark brown pole (diameter 1.7 mm).

Advertisement call. Recordings were taken in captivity by P. Klaas from specimens of unknown origin (no vouchers preserved; living specimens determined by F. Glaw). Quality of recordings was poor due to echo effects in the aquarium. However, it could be ascertained that the general call structure was similar to that of *S. spinosa*. Call duration was between 1799 ms and 12 492 ms, and calls were composed of 45-317 notes (n=7).

Some of the calls with the longer duration were interrupted by 1-2 short pauses of 258-326 ms. Note duration was ca. 35-38 ms, duration of intervals between notes 7-19 ms. Note repetition rate was 25/s. Frequency was 400-1000 Hz; a number of irregular harmonics were visible on the sonagram but were probably artifacts caused by the recording conditions.

Distribution. Only known from two specific sites in the Fierenana region, central eastern Madagascar (Fig. 3): (1) Ampahanana, and (2) Sahanomanana.

Comments. This species is assigned to the genus *Scaphiophryne* based on absence of vomerine teeth, rudimentary maxillary teeth, clavicula, small cartilaginous sternum, large cartilaginous omosternum (verified in ZSM 645/2000 by dissection), tarsal tubercle, widely distensible single subgular vocal sac, and high overall phenetic similarity to *Scaphiophryne spinosa* and *S. marmorata*. The pairwise genetic divergences, in a portion of the 16S rRNA gene, of *Scaphiophryne boribory* compared with the other two species of the *Scaphiophryne marmorata* complex were: 3.1% (17 substitutions) in comparison to *S. marmorata*, 2.4% (13 substitutions) in comparison to *S. spinosa*. The lowest differentiation was found in comparison with a fourth green-coloured species, *S. madagascariensis* (1.8%; 10 substitutions). Differentiation with the remaining species of *Scaphiophryne* was 1.8-4.4% (20-24 substitutions; Table 2). No phylogenetic analysis was performed because of the low amount of informative sites (see Vences et al., 2002*b*).

KEY TO ADULT *SCAPHIOPHRYNE*
WITH EXPANDED TERMINAL FINGER DISCS

1a. Dorsal colouration white with green, black and pink markings; ventral surface uniformly whitish; only known from the Isalo region — *Scaphiophryne gottlebei*

1b. Dorsal colouration always without pink, ventral surface with black or brown — 2

2a. Large species (SVL 47-60 mm); dorsal skin with few large tubercles in life, usually smooth in preservative; tympanum always concealed; ventral surface black with white spots — *Scaphiophryne boribory*

2b. Usually smaller (SVL 32-49 mm); dorsal skin with distinct tubercles in life and in preservative; tympanum concealed or visible; ventral surface light with dark marbling or with dark markings at least on the chest — 3

3a. SVL up to 49 mm, but often smaller than 40 mm; dorsal skin with tubercles of relatively regular size; no large and spiny tubercles above forelimbs and in tympanic region; tympanum, if visible, larger than 50% of eye diameter; posterior belly usually light with dark marbling — *Scaphiophryne marmorata*

3b. SVL 40-48 mm; dorsal skin with a large number of irregularly sized spines and tubercles; large spiny tubercles above forelimbs, at maxilla commissure, and in the tympanic region; tympanum, if visible, smaller than 50% of eye diameter; posterior belly usually uniformly light — *Scaphiophryne spinosa*

DISCUSSION

Previous workers (e.g. Noble & Parker, 1926; Guibé, 1978) considered *Scaphiophryne marmorata* to belong to a genus different from *Pseudohemisus*, which included the taxa *madagascariensis, brevis* and *calcarata* (and their synonyms). Blommers-Schlösser & Blanc (1991) noted that other than the expanded finger discs of *S. marmorata*, there were no characters to maintain this classification, and they lumped all species in the genus *Scaphiophryne*. Busse & Böhme (1992), describing a second species with expanded finger disks (*S. gottlebei*), proposed that the taxa sharing this character be considered as the subgenus *Scaphiophryne,* and the species with non-expanded disc as the subgenus *Pseudohemisus.* Until the phylogenetic relationships of these frogs are better clarified, we do not consider a subgeneric division to be useful.

The genus *Scaphiophryne* was listed by Vences *et al.* (2000*a*) as a relict genus of low species diversity having a reproductive mode adapted to savanna areas of western Madagasscar. At this time, a total of six nominal species of *Scaphiophryne* were known, three of which were endemic to arid western Madagascar, two to high-altitudes, and only one (*S. marmorata*) known from the rainforests of eastern Madagascar.

TABLE 2. Pairwise distances (total number of substitutions; indels not counted) of species of *Scaphiophryne* in a fragment of c. 540 base pairs of the mitochondrial 16S rRNA gene.

		1	2	3	4	5	6	7
1	*S. calcarata*	-						
2	*S. brevis* (Kirindy)	24	-					
3	*S. brevis* (Ifaty)	23	1	-				
4	*S. gottlebei*	24	25	24	-			
5	*S. madagascariensis*	22	22	23	17	-		
6	*S. spinosa*	23	18	17	15	12	-	
7	*S. marmorata*	26	20	20	20	15	18	-
8	*S. boribory*	24	21	21	20	10	13	17

The present review implies some modifications to this view. The recognition of *Scaphiophryne boribory* and *S. spinosa* as distinct species elevates the number of species known from eastern rainforests to three. Not counting the dubious names *S. obscura* and *S. verrucosa*, the genus *Scaphiophryne* currently contains a total of seven nominal species, but this does not reflect adequately its diversity. At least three additional species have already been identified by us. Nevertheless, the actual diversity of *Scaphiophryne* is distinctly lower than that of the most diverse endemic Malagasy frog radiations. If the three taxonomically unrecognized species are taken into account, currently three species of *Scaphiophryne* appear to be endemic to eastern rainforests, four species to western Madagascar, two species to high elevations, and one species (*S. marmorata*) is distributed in the east as well as in the west. In contrast to the mantellid and cophyline radiations, which clearly have their maximum diversity in humid eastern Madagascar, *Scaphiophryne* appears to have diversified to an equal extent in the eastern, central and western biogeographic regions of the island.

An important feature of *Scaphiophryne* appears to be their low differentiation in advertisement calls and reproductive biology. We herein provide evidence that the general temporal and spectral pattern of the calls of *S. spinosa* and *S. boribory* – which are sympatric in the Fierenana region – are similar. The call of *S. madagascariensis* from the Ankaratra Massif as described by Vences *et al.* (2002*b*) also shows the same structure, while the calls of *S. brevis, S. calcarata* and and *S.* sp. from the Kirindy forest differ from this pattern and from each other (F. Glaw, unpublished). However, the reproductive biology of all these species appears to be similar (Blommers-Schlösser, 1975; Glaw & Vences, 1994; Vences *et al.*, 2002*b*): a short and explosive bout of reproductive activity follows heavy rainfall; specimens forming extremely loud choruses in stagnant, mostly temporary waters, into which the many small eggs with dark pigment are laid. The fact that partly sympatric species of low differentiation in reproductive mode do not show distinct differences in advertisement calls is exceptional among Malagasy amphibians, and will certainly deserve further investigation in the future.

The conservation of *Scaphiophryne marmorata, spinosa* and *boribory* will probably depend on the protection of suitable primary habitat. Their ability to survive in degraded habitats is uncertain (unlike other species such as *S. brevis* and *S. calcarata)*. Both *S. spinosa* and *S. marmorata* occur in protected areas in Madagascar. However, *S. boribory* is known only from specific sites in the Fierenana region, outside the protected area network, in areas of highly active ongoing deforestation. Possibly this species also occurs in the Mantadia (=Mantady) or Perinet (= Analamazaotra) reserves in the Andasibe region, but herpetological surveys have yet to confirm this. More survey effort in these regions, and more generally in the forest corridor between Mantadia and Zahamena, would be especially valuable for better determining the distribution limits of this species.

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POPULATION DYNAMICS OF THE EUROPEAN LEAF-TOED GECKO (*EULEPTES EUROPAEA*) IN NW ITALY: IMPLICATIONS FOR CONSERVATION

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A continental population of the European leaf-toed gecko (*Euleptes europaea*) was studied from 1996 to 2000 in Central Liguria (NW Italy), to obtain data on demography, abundance and population trends. Each year in July, three night mark-recapture sessions were carried out to obtain data on population structure and abundance. The population structure of the European leaf-toed gecko was assessed on the basis of polymodal body-size frequency distributions, and the demographic structure was characterized by three well-separated size groups: juveniles ending their first year of life, subadults in their second year and mature individuals aged three or more. Growth appeared constant during the first two years, and then apparently decreased in the third year when functional sexual maturity was achieved in both sexes. The sex ratio of the reproductive population did not differ from unity and females were, on average, 5% larger than males. The physiological status of different age groups, assessed by a body condition index, did not show significant variations during the study. The observed stability of overall population size highlights the capacity of small and completely isolated colonies of the European leaf-toed gecko to persist for a long time, even in extremely simple habitats. However, the number of breeding adults showed large fluctuations, from 120 to 50 animals in different years, and count data suggested a downward trend in population size. Nevertheless, the statistical power of not rejecting the null hypothesis of "no change" (i.e. Type II error) was low, and a Monte Carlo simulation analysis showed that doubling the monitoring effort (i.e. 10 annual surveys with 30 sampling occasions) should be sufficient to detect a 10% decrease in population size with a power of >0.80. Without detailed population data from such intensive monitoring, isolated populations of *E. europaea* remain exposed to a high risk of extinction through stochastic and anthropogenic processes.

Key words: conservation, Geckonidae, Liguria, population size, population structure, power analysis

INTRODUCTION

Geckos are relatively poorly studied compared to some other lizards (Henle, 1990*a*), and little is known about their population ecology and demography in the Mediterranean basin. In this area, out of 41 gecko species (Sindaco, 1998), only the European leaf-toed gecko, *Euleptes europaea* (Gené, 1839), formerly *Phyllodactylus europaeus* (see Bauer *et al.*, 1997), is listed as "vulnerable" by IUCN (1996). It is an extremely small nocturnal lizard with a maximum adult body mass of 2 g (Delaugerre, 1997); it is endemic to the western Mediterranean, and belongs to a monophyletic genus (Bauer *et al.*, 1997). *Euleptes europaea* is found on the mainland and some islands of Southern France; the coast and two islets of Liguria (NW Italy); the coast of Tuscany and the Tuscan Archipelago; Sardinia and Corsica and nearly all their satellite islands, as well as three islands off the coast of Tunisia (Delaugerre, 1997). Its present geographical range is clearly relictual and is composed of hundreds of isolated populations inhabiting islets and a few continental areas in Southern France, Liguria and Tuscany (Capocaccia, 1956; Vanni

& Lanza, 1978; Delaugerre, 1981*a*, 1997). *Euleptes europaea* shows peculiar biological features, being particularly well adapted to small islands and even islets of less than 1000 m² in size, where it is the only resident vertebrate and the main terrestrial predator (Delaugerre & Cheylan, 1992; Poggesi *et al.*, 1996; Thibault, *et al.*, 1987). Across its geographical range, *E. europaea* displays a certain degree of morphological variation in body size, sexual size difference and coloration, suggesting that environmental or stochastic events have had a direct influence upon the ecological adaptation and life history of these populations (Delaugerre, 1985, 1992; Kulesza *et al.*, 1995).

Understanding geographical and temporal variations in life-history characteristics appears critical for the conservation of gecko populations living in fragmented habitats (Sarre, 1995, 1998; Sarre *et al.*, 1996). Although *E. europaea* is considered by the IUCN to be "vulnerable" (IUCN, 1996) and by the European Herpetological Society to be "threatened" (Stumpel *et al.*, 1992), little is known about its population dynamics and long-term fluctuations in abundance. Whereas Delaugerre and co-workers (Delaugerre, 1981*a,b*, 1985; Delaugerre & Dubois, 1986; Delaugerre & Cheylan, 1992) studied in detail some aspects of its population autoecology in southern France and Corsica, quantita-

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tive data on Italian and Sardinian populations are completely lacking, apart from a preliminary report by Allosia *et al.* (1999) on a continental population. For these reasons, the conservation actions proposed by Stumpel *et al.* (1992) were based on little field evidence.

The purpose of this study was to collect data on the demography, abundance and population trend of an isolated continental population of *E. europaea,* to evaluate its conservation status, to propose a long-term monitoring protocol to be used in similar ecological situations and to provide a baseline for comparison with other populations.

STUDY SITE AND METHODS

The study site is located near Genova, NW Italy, at an altitude of about 300 m a.s.l. The climate of this region is sub-Mediterranean (*sensu* Daget & David, 1982) with a short dry period in July and two rainfall peaks in October and March (Brancucci, 1994). Mean annual temperature averages 17 °C (ranging from 21-27 °C), reaching a maximum (24°C) in July-August. Geckos were found only on the walls of an isolated building that is now abandoned. This isolated population was first described by Capocaccia (1956) and is considered by several authors to be of natural origin (Capocaccia, 1956; Delaugerre, 1981*a*; Vanni & Lanza, 1978; Sindaco, 1994); however, an introduction by man cannot be completely ruled out. During this study, three other reptile species were observed at the study site: one specimen of the common wall lizard (*Podarcis muralis*), and one adult wall gecko (*Tarentola mauritanica*) were seen on the building at dusk in 1996 and 1999 respectively, while the Southern smooth snake (*Coronella girondica*) a potential gecko predator, was observed on the walls of the building by night once in 1997, twice in 1998 and once again in 1999.

The gecko population was sampled from 1996 to 2000 in July, during relatively hot nights, by at least three researchers. Three sampling sessions were performed within a time period not exceeding eight days. Specimens were captured from ground level up to 2.5 m, since few geckos were observed above this height. The total wall surface sampled in this study was about 150 m². Intensive collecting started immediately after sunset and continued for about 45 min after the first gecko was captured, or until two complete searches on the building walls failed to locate further specimens. Animals were spotted by the aid of hand- and head-lights, captured by hand and kept temporarily in small plastic boxes. Snout-vent length (SVL) was measured to the nearest mm, from the tip of the snout to the posterior end of the vent, holding the gecko ventral-side-up against a transparent plastic ruler. Body mass of individuals with complete tails (W) was measured, to the nearest 0.1 g, with a Pesola spring dynamometer. Geckos were sexed on the basis of external sexual characters: presence of horny spurs on both sides of the tail base in males, absence of spurs and presence of well developed white neck glands in females (Camerano, 1904; Delaugerre, 1981*b*).

Moreover, geckos were inspected for recently broken tails (i.e. tails with missing parts) and developing eggs. All the animals captured on the same night were marked with a single, specific paint spot on the back, and different colours were used in successive sampling sessions. At the end of each night's search, all captured geckos were released on the study site.

The SVL measurements generated polymodal frequency distribution histograms that were analysed with the FAO-ICLARM Stock Assessment Tools (FiSAT) computer programme (Gayanilo *et al.*, 1996). This programme enables the decomposition of mixed length-frequency distributions into their Gaussian components by means of Bhattacharya's (1967) log-differences method. This is an iterative process in which each identified component is subtracted from the remainder of the sample using a Gaussian function. A linear regression analysis for each separate component and a χ^2 goodness-of-fit statistic for the entire sample is then calculated (classes with expected frequency <5 are automatically combined with adjacent classes). In addition, a separation index (S.I.) for each pair of adjacent groups is estimated; when S.I. ≤ 2, separation between components is unreliable (Sparre & Venema, 1996). Moreover, FiSAT provides the mean, the standard deviation, and the theoretical number of individuals in each group. As *E. europaea* lives for up to eight years in captivity, according to Knoepffler (1973), there was the possibility of recapturing the same individuals in successive years. For this reason, between-year variations in population structure, mean SVL, mean body mass, and growth increments were analysed by Friedman two-way analysis of variance by ranks, a nonparametric test used to compare repeated measures of matched groups (Siegel & Catellan, 1988). Mean SVL and body mass of males and females within each year-sample were compared by means of Mann-Whitney nonparametric tests. Statistical analyses were processed using Minitab release 12.21 computer software. The population abundance was estimated by means of the CAPTURE programme (White *et al.*, 1982), which is suitable for closed populations. Three estimators were used, the Null Model (M_0), in which capture probabilities for every animal are assumed to be constant on every capture occasion; the Jacknife Model (M_h), which assumes that capture probabilities vary between animals; and the Darroch Model (M_t), which allows capture probabilities to vary only by time – thus, for 't' capture occasions, 't' different values of capture probability are calculated (White *et al.*, 1982). All models give the estimated population size, standard error, mean capture probabilities, and 95% confidence intervals.

The physiological status of immature individuals, sub-adults, females and males was assessed separately by means of a body condition index (BCI), calculated as the residual obtained from a regression of log-transformed body mass against log-transformed SVL (Henle, 1990*b*). Specimens with broken tails and egg-carrying females were excluded from calculations.

The population trend of count data was assessed with the Spearman rank-order correlation coefficient (Siegel & Catellan, 1988). A power analysis was conducted by means of MONITOR version 6.2, a programme designed to test the power of monitoring protocols to detect linear or exponential trends in count data (Gibbs, 1995). In this software, the user defines the number of annual surveys and the mean and standard deviation of population counts. MONITOR uses Monte Carlo simulations to generate data sets based on these input variables, estimating how often the sampling protocol will detect trends of varying strength (Gibbs, 1995). The statistical power estimated ranges from 0 (i.e. low power) to 1 (i.e. high power), and was used to determine how many years of data are required to provide statistical confidence in the results (Reed & Blaustein, 1995). The coefficient of variation (CV = standard deviation/mean) was used to evaluate the variability of the population size estimates and field count data.

RESULTS

INFLUENCE OF AIR TEMPERATURE ON ACTIVITY

On all sampling occasions, gecko activity started shortly after sunset. During our capture-recapture sessions, air temperatures varied from 18.5 to 25 °C (mean 21.9, SD±2.2, n=15). There was no correlation between air temperature and the number of geckos captured during the study (Spearman rank correlation test r_s=0.075, n=15, P=0.71).

POPULATION STRUCTURE

During this study 318 geckos were measured (Table 1). As leaf-toed geckos are long-lived and marks were not permanent, this number probably does not correspond to different individuals. The smallest individuals were 20 mm in SVL, and the largest was a 46 mm female. In all year-samples, size-frequency distributions were polymodal and were resolved into three Gaussian components (Fig. 1). Since in all cases S.I. was > 3.2, the separation between adjacent components was considered reliable. These components were estimated to correspond to three age classes on the basis of external
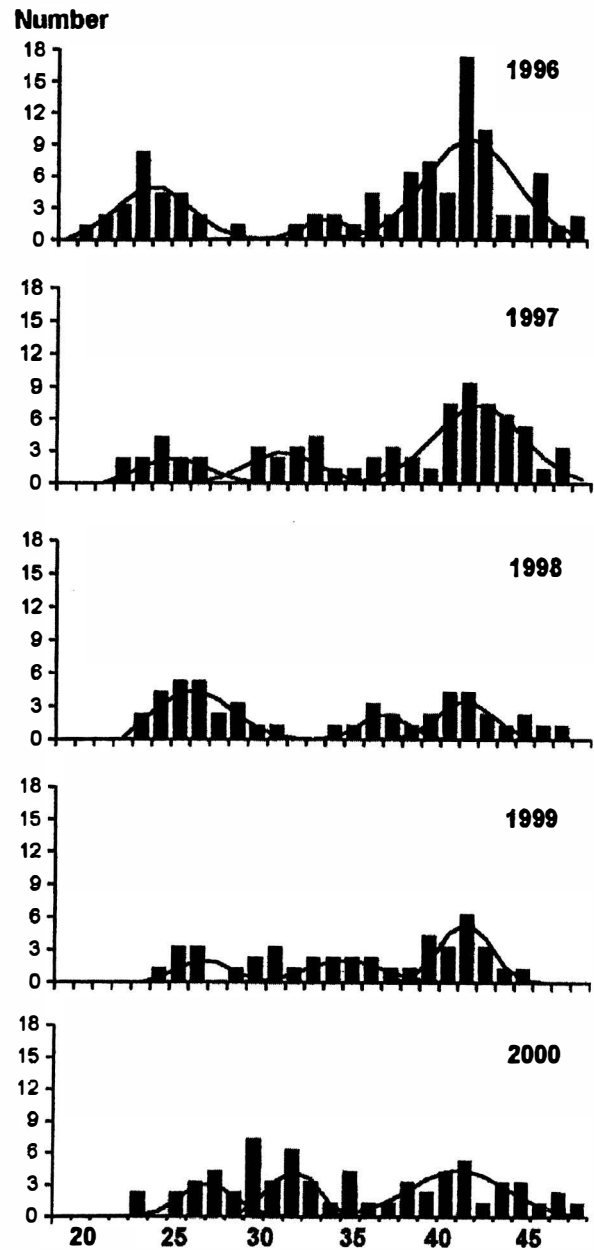

FIG. 1. *Euleptes europaea* body size distributions. SVL = snout-vent length. The curves separating each component were calculated by the FiSAT software (see text).

TABLE 1. Population structure of *Euleptes europaea* from central Liguria, estimated by the FiSAT computer programme (Gayanilo *et al.*, 1996). N, geckos caught; N_{th}, theoretical number of geckos in each snout-vent (SVL) component. NS, P>0.10.

| Year | N | Component 1 | | Component 2 | | Component 3 | | χ^2 |
		N_{th}	mean SVL ±SD	N_{th}	mean SVL ±SD	N_{th}	mean SV ±SD	
1996	92	24	23.3±1.9	6	32.5±1.2	57	40.1±2.4	χ^2_{10} = 15.1 NS
1997	72	10	24.5±1.7	13	30.1±1.8	43	40.3±2.3	χ^2_{2} = 4.0 NS
1998	49	22	25.6±1.9	7	35.5±1.1	13	39.7±1.4	χ^2_{4} = 0.7 NS
1999	41	7	26.2±1.3	10	33.5±1.9	17	39.7±1.2	χ^2_{3} = 2.4 NS
2000	64	11	26.5±1.4	18	31.5±2.1	23	39.8±2.7	χ^2_{6} = 2.2 NS

TABLE 2. Sexual dimorphim in SVL (mm) and body mass (g) in the reproductive population of *Euleptes europaea*.

Year	Sex	SVL±SD (N)	SVL range (min-max)	W±SD (N)
1996	Females	41.7±2.62 (24)	37-46	1.4±0.21 (23)
	Males	39.1±1.90 (36)	36-43	1.1±0.15 (34)
1997	Females	41.4±2.19 (26)	36-45	1.6±0.28 (23)
	Males	38.6±3.01 (21)	35-43	1.2±0.21 (16)
1998	Females	40.7±2.42 (12)	38-45	1.5±0.15 (11)
	Males	39.4±1.27 (7)	37-40	1.2±0.18 (6)
1999	Females	40.9±1.35 (7)	39-43	1.6±0.17 (7)
	Males	39.2±1.08 (11)	38-41	1.2±0.20 (7)
2000	Females	41.5±3.26 (13)	36-46	1.4±0.28 (11)
	Males	39.6±1.76 (13)	37-43	1.4±0.14 (10)

sexual characters (Delaugerre, 1981*b*; Delaugerre & Dubois, 1986): the smallest size-group (SVL $\leq$ 30 mm) was composed of immature specimens (i.e. without mature gonads and external sexual characters); the intermediate size-group (31 $\leq$ SVL $\leq$ 33-36 mm, depending on the year sample) comprised subadults with maturing gonads but with poorly developed external characters (as males had very small horny spurs and females were never gravid). The largest size class (SVL $\geq$ 33-36 mm) comprised only mature specimens of both sexes. This demographic composition did not differ among years, either by estimated number (Friedman's test by year blocked by age group S=2.13, df =4, P=0.71) or by mean SVL (Friedman's test by year blocked by age group; S=0.47, df=4, P=0.97, adjusted for ties). The mean growth increment between the first

and second size classes was 7.40±2.15 mm (range 5.0-9.9 mm) and the mean growth increment between the second and third size classes was 7.30±2.25 mm (range 4.2-10.2 mm). These growth increments did not vary statistically during the study period (Friedman test by year blocked by age group: S=0.80, df=4, P=0.930) or between age classes (Friedman test by age group blocked by year: S=0.20, df=1, P=0.655).

SEXUAL DIMORPHISM

For mature males as well as for mature females, no significant differences between years were detected in mean body length or mass (Table 2; Friedman test by year blocked by sex: S=4.60, df=4, P=0.406 and S=2.40, df=4, P=0.663 for females and males, respectively).

Females were significantly larger than males in 1996, 1997 (Mann-Whitney U-test; P=0.001 in both cases) and 1999 (P=0.02), but not in 1998 and 2000 (P=0.10). Sexual dimorphism was, on average, 5% – ranging from 7% both in 1996 and 1997, to 3% in 1998. The sex ratio of the adult population fluctuated from 0.67 females per male in 1996 to 1.72 females per male in 1998 (Table 2).

BODY CONDITION INDICES

The mean BCI was calculated for juveniles, subadults, adult males and adult females separately (Table 3). Standard deviations were usually large, indicating strong intra-group variability. Annual BCI values were compared by means of Friedman's two-way analysis of variance by year blocked by age group and did not show significant variations (S=6.97, df=4, P=0.137, adjusted

TABLE 3. *Euleptes europaea* mean body condition index (BCI). Specimens with broken tails and females with eggs were excluded.

Year	Juveniles		Subadults		Adult females		Adult males	
	N	BCI±SD	N	BCI±SD	N	BCI±SD	N	BCI±SD
1996	19	-0.07±0.21	8	-0.04±0.18	34	-0.10±0.09	12	-0.03±0.09
1997	11	0.19±0.20	13	0.12±0.13	16	0.05±0.11	23	0.03±0.07
1998	22	-0.04±0.22	6	0.13±0.05	6	0.01±0.05	7	0.03±0.15
1999	7	0.15±0.08	15	-0.09±0.21	10	0.04±0.10	6	0.01±0.14
2000	13	-0.08± 0.20	23	-0.09±0.12	7	-0.05±0.06	10	0.12±0.09

TABLE 4. Number of juvenile, sub-adult and adult female and male *Euleptes europaea* with complete or recently broken tails.

Year	Juveniles		Subadults		Females		Males		Total (%)	
	complete	broken	complete	broken	complete	broken	complete	broken	complete	broken
1996	20	4	8	-	23	1	34	2	85 (92)	7 (8)
1997	11	1	13	-	23	3	16	5	63 (88)	9 (12)
1998	22	1	6	1	11	1	6	1	45 (92)	4 (8)
1999	9	1	11	-	8	-	11	-	39 (98)	1 (3)
2000	19	1	17	1	11	2	11	2	58 (91)	6 (9)
Total	91	8	55	2	70	7	78	10	290 (91)	27 (9)

for ties). In addition, age groups displayed similar mean BCI (Friedman test by age blocked by year: $S=0.60$, df=3, $P=0,896$, adjusted for ties), indicating an overall similar body condition within each year-sample.

The numbers of geckos with intact and recently broken tails are shown in Table 4; differences among years (Friedman test by year blocked by age group: $S=5.59$, df=4, $P=0.232$, adjusted for ties) and among age classes (Friedman test by age group blocked by year: $S=5.12$, df=3, $P=0.163$ adjusted for ties) were not statistically significant, suggesting that the agents causing tail loss (i.e. predator attacks or intraspecific competition) did not vary in their intensity during the study period.

POPULATION SIZE

During our five-year study only four marked juveniles were recaptured (i.e. one in 1997 and three in 2000), suggesting a differential response to manipulation between juveniles and adults, as already observed by Delaugerre (1985) in the population living on the Corsican islet of Sperduto Grande. The percentage of subadults plus adults recaptured once varied from 22% in 2000 to 15% in 1998, with an average value of 18%.

There were slight differences (on average, 10%) among the estimated population sizes obtained using different methods (Table 5), and capture probabilities were on average higher than 0.20, a value considered adequate in estimating abundance in closed populations (White *et al.*, 1982). Estimated probabilities of capture were similar among models, and those calculated by Darroch's estimator did not show significant variations among years (Friedman test by year blocked by capture occasion: $S=2.10$, df=4, $P=0.717$, adjusted for ties). The adult subpopulation was characterized by large fluctuations in estimated abundance, ranging from 120
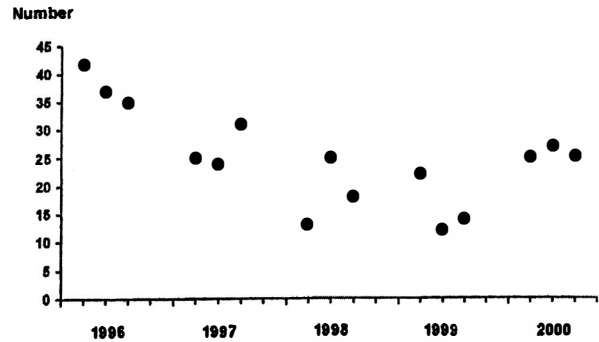

FIG. 2. Temporal variations in capture data in a *Euleptes europaea* population from central Liguria.

in 1996 and 1997 to 50 in 1998 (Table 5). The CV showed relatively low variations according to the model used and was 0.33 (Null estimator), 0.35 (Darroch estimator) and 0.41 (Jacknife estimator). Estimated gecko densities varied from 0.8 to 0.4 individuals per square metre of wall surface.

POPULATION TREND

The number of individuals captured during each survey, including juveniles, was used to evaluate population trends over time. The resulting trend was downward (Fig. 2), but not yet statistically significant ($r_s=-0.46$, $P>0.06$, $N=15$). The CV of population counts was 0.35.

The mean (25.0) and the standard deviation (±8.8) of field count data were used as input variables to test the statistical power. Results of 10 000 simulations showed that our five-year study had a low power (0.22) to detect a 10% linear decrease in *E. europaea* abundance ($\alpha=0.05$, two tailed). With our monitoring protocol, 10 annual surveys with a total of 30 population counts would be needed to obtain evidence of a 10% decrease

TABLE 5 *Euleptes europaea* population size estimated by the CAPTURE programme (White *et al.*, 1982). N_e, number estimated; SE, standard error; capt. prob., capture probabilities; CI, confidence intervals.

Estimator	1996	1997	1998	1999	2000
NULL (M_0)					
N_e	117	123	52	69	95
SE	17.56	23.98	13.73	19.31	23.81
Capture P	0.25	0.20	0.22	0.19	0.19
95% CI	93-164	91-189	37-97	47-129	66-165
DARROCH (M_t)					
N_e	116	120	48	66	94
SE	17.18	22.61	11.58	17.31	22.67
Capture P 1st	0.28	0.18	0.12	0.29	0.14
Capture P 2nd	0.23	0.17	0.35	0.15	0.21
Capture P 3rd	0.25	0.28	0.25	0.17	0.21
95% CI	93-162	90-182	36-85	46-119	66-140
JACKNIFE (M_h)					
N_e	133	113	48	61	77
SE	10.64	10.00	6.57	7.58	8.29
Capture P	0.25	0.23	024.	0.20	0.23
95% CI	115-156	97-136	39-65	50-80	65-97

with a statistical power >0.80 (α=0.05, two-tailed), which is considered adequate in long-term monitoring protocols (Peterman, 1989; Meyer et al., 1998). Similar results were obtained for exponential population trends and therefore are not shown.

DISCUSSION

During our study, gecko activity was not related to air temperature and the observed fluctuations have to be attributed to other factors such as wind speed, relative air humidity, food availability or, more probably, to a combination of environmental and behavioural factors. Indeed, this species shows a weak dependence on air temperature for its activity, having one of the better thermoregulatory abilities among Mediterranean geckos (Delaugerre, 1984). For this reason, E. europaea forages even on cool nights, and is capable of surviving up to about 1500 m a.s.l. in Corsica and 1300 m a.s.l. in Sardinia (Delaugerre, 1984, 1992). Thus, the observed irregular pattern of activity in relation to ambient temperature suggests that this species may be difficult to find, even in apparently suitable conditions.

The demographic structure observed in the NW Italian population was similar to that of conspecifics on the islands of Port-Cros (Delaugerre, 1981b) and Lavezzi (Delaugerre, 1984), in which three age-groups were recognized. In Port-Cros Island (S France), newborn individuals hatch from August until September at about 17 mm SVL (Delaugerre, 1981b). Thus, in the study population, juveniles in the smallest age-class were probably ending their first year of life, subadults were becoming two years old, and adults were in their third year or older. Males with well developed tail spurs and gravid females were found only in the largest body-size group, suggesting that functional sexual maturity was reached by both sexes during the third year of life or later. Growth was constant during the first two years. Several demographic parameters such as age structure, sex ratio, immature annual growth, and physiological condition seemed to remain constant during the five-year study period. The only comparable data on temporal variation in this species are those concerning adult sexual dimorphism reported by Delaugerre & Dubois (1986) in the Port-Cros population (SW France). These authors analysed the mean body length of males and females collected in different seasons during two successive years, and concluded that they were relatively constant. Our results confirm these observations, extending them to more complex biological features such as overall demographic composition and physiological status. However, caution should be used in interpreting these data, since E. europaea is long-lived, and the persistence of old individuals in successive years could influence these findings.

Adult capture probabilities did not differ among years but were relatively low, considering that the habitat is physically constrained and very easy to explore all over. Juveniles displayed a high behavioural avoidance of recapture, suggesting that they were in some way disturbed by spotlights and/or manipulation. Indeed, the extremely low number of first-year individuals recaptured within one annual sampling indicates that this extremely small and delicate age class (mean body mass = 0.4 g) was in some way disturbed by the investigators. Moreover, this gecko had an irregular pattern of activity and only a fraction of the adult population was active each night, a behavioural pattern already observed in the tropical gecko Lepidodactylus lugubris, in French Polynesia (Ineich, 1988).

While demographic and physiological parameters seemed relatively constant from year to year, population size estimates showed large fluctuations during the study period. These results should be regarded with some caution, since fluctuations could be due to both extrinsic (e.g. variations in food availability or predation pressure) and behavioural factors (e.g. avoidance response to capture and searching disturbance). In any case, the statistical power to reject the null hypothesis of no change in population size (i.e. Type II error) was low, and a simulation analysis showed that extending the monitoring protocol up to 10 years should ensure adequate power to detect a biologically significant decrease in population size.

Euleptes europaea is characterized by its nocturnal life-style, small body size, long life expectancy, and aggregation behaviour. Thus, it should be able to maintain populations of sufficient size in even small and simplified habitats, like islets (Delaugerre & Dubois, 1986), as is the case of several insular populations studied in Corsica (Delaugerre & Cheylan, 1992). However, in the absence of a metapopulation structure, E. europaea remains exposed to a high risk of local extinction. In fact, several historical occurrences of population decline or possible extinction are known of Southern France islets. For instance, the species was discovered, considered extinct, and then rediscovered on the Marseilles' islets of Pomègue and If (Mourgue, 1910a, 1924, 1930; Philippe, 1955 ; Delaugerre 1981a; Aillaud & Bayle, 1996). On the two islets of Grand and Petit Congloué (Marseilles), where it was discovered by Mourgue (1910a), it has not been sighted since that time, despite several diurnal and nocturnal searches (Nougaret & Petenian, 1999; M. Cheylan, in litt.). It is now considered extinct (Delaugerre, 1981a) on the islet of Bandor (Bandol), where it was first discovered by Mourgue (1910b) and then confirmed by Jahandiez et al. (1933). If there is no misunderstanding concerning the designation of the islet, this gecko might also be extinct on the îlot de Pendus (Marseilles), where it had been discovered by Blanc (1876, 1878), considered extinct by Mourgue (1910a) and then by Delaugerre (1981a). Moreover, the present fragmented continental distribution demonstrates that in the past, many continental extinctions occurred, reducing the species' distributional range to hundreds of small, favourable habitat patches. In these environments, the long-term persistence of gecko populations will be strongly affected by habitat alteration, food shortage, epidemics, increase of

predation pressure and catastrophic climatic events. For these reasons, the conservation status of the European leaf-toed gecko all over its distributional range should be assessed to evaluate strategies for population conservation. *Euleptes europaea* is a species listed on annexes II and IV of the European Union Council Directive No. 92/43/EEC on the Conservation of Natural Habitats and of Wild Fauna and Flora (the "Habitats and Species" Directive); thus it is protected and requires the establishment of Special Areas of Conservation (SAC) that will form part of the Europe-wide Natura 2000 network. Thus, the assessment of *E. europaea* population status in all the other SACs – in S France, Corsica, Liguria, Tuscany, Sardinia – should be undertaken, and member states should co-operate to ensure consistency of monitoring methods. The monitoring protocol used in the present study – based on simple counts of captured individuals – needed a total sampling effort of about nine nights per person per year, and was rather inexpensive and easy to implement, at least for a small isolated population. Thus, it could be used by trained operators in other, similar ecological situations to evaluate the status and the ongoing trend in other isolated populations.

ACKNOWLEDGMENTS

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LARVAL GROWTH PLASTICITY IN WILD POPULATIONS OF THE BETIC MIDWIFE TOAD, *ALYTES DICKHILLENI* (ANURA: DISCOGLOSSIDAE)

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We studied plasticity in larval development and growth in the endangered toad *A. dickhilleni* in south-eastern Spain. The species is strongly dependent upon artificial breeding sites. The relation between growth and differentiation of larvae is variable across breeding sites. Tadpoles in fountains showed a linear growth/development relationship ("fast trajectory"), whereas in streams and tanks the relationship was non-linear("slow trajectory"). This variation is interpreted as developmental plasticity that allows the species to maximize the range of breeding habitats colonizable in areas characterized by scarce and unpredictable precipitation, as occurs across most of its distribution.

Key words: amphibian conservation, development, reproduction, Spain, tadpoles

INTRODUCTION

Phenotypic plasticity and environmental heterogeneity are subjects of interest in modern evolutionary biology (Berven & Gill, 1983; Via & Lande, 1985; Relyea, 2001). The different phenotypes a single genotype can produce under certain conditions enhance survival probabilities of organisms that live in unpredictable environments with differential environment-dependent responses. Comprehensive studies about phenotypic plasticity should start, according to Newman (1988*a,b*), with preliminary identification of selective environmental factors, variable in both a temporal and a spatial scale, and phenotypic variability in morphological traits. The next step concerns the study of the possible relationships between this phenotypic variability and the differential fitness of each variant under each distinct set of environmental conditions. Eventually, quantitative genetic analyses of different cohorts within a single species can provide an evaluation of the heritability of the traits studied, which can be used to estimate the extent to which these can be affected by natural selection.

Amphibian populations inhabiting areas characterized by scarce, highly unpredictable precipitation have proved to be good models for studying genotype-environment interactions (Newman, 1988*a,b*, 1989, 1994; Tejedo & Reques, 1994; Reques & Tejedo, 1997). This paper describes phenotypic variability in developmental traits of the endangered Betic midwife toad, *Alytes dickhilleni*, which is endemic to a few mountain ranges of the south-eastern Iberian Peninsula. In the karstic limestone mountains inhabited by *A. dickhilleni*, water availability for reproduction is limited, discontinuous and unpredictable both within and between years, being in most cases restricted to human-made water containing constructions such as water tanks, fountains or troughs (Antúnez *et al.*, 1982; García-París & Arntzen, 1997; own data). This, considering the relatively long larval period of the species, which is a general condition in the genus *Alytes* (Crespo, 1982), imposes problems for the survival of *A. dickhilleni* in these regions. Phenotypic variability in larval traits is explained in this context as a product of different developmental strategies that would result in a better performance in the population-environment interaction.

We have studied the reproductive phenology and larval development of *A. dickhilleni* in the northernmost mountain range within its distribution area, the Sierra de Alcaraz, in the Province of Albacete (Castilla-La Mancha, south-eastern Spain), where the species maintains one of the largest and healthiest groups of populations overall. We followed the development of larvae of *A. dickhilleni* from hatching to metamorphosis in a selection of breeding sites (artificial and natural) and analysed the relationship between growth and differentiation across sites.

MATERIALS AND METHODS

We found evidence of reproduction of *A. dickhilleni* at 65 sites across the study area. As cited for Sierra Tejeda populations (Antúnez *et al.,* 1982), *A. dickhilleni* populations from the Sierra de Alcaraz mainly use three types of breeding sites, two of which are related to human activities and constitute more than 50% of the total number of sites used (Table 1). Brooks are temporary or permanent courses of water whose duration is related to each year's weather conditions and varies both within and between years. Occasionally they are associated with artificial dams that can hold water throughout the year. Fountains have a constant, low water level for most of the year, but sometimes they dry out or are emptied for cleaning. Finally, water tanks are relatively stable aquatic environments that have a constant, high level of water during most of the breeding period, but they are also emptied at least once a year for cleaning, which makes them unpredictable environments.

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TABLE 1. Breeding site categories and their properties as aquatic systems used for amphibian reproduction (see explanations in text).

Type	Stability	Predictability
Water tanks	Intermediate	Low
Brooks	Low	Low
Fountains	High	Intermediate

At some breeding sites sampling was carried out once a month from August 1995 to October 1996. Those selected included representatives of the three categories most commonly used by *A. dickhilleni*: brooks, water tanks and fountains. We chose two sites of each type: brooks (Rio Turruchel and Arroyo del Saz), water tanks (Venta Mendoza and El Bellotar) and fountains (Las Raigadas-La Guitarra and Riópar Viejo). On each visit larvae were captured by dipnetting. Total length (TL), snout-vent length (SVL) and developmental stage (GSD), following Gosner (1960), were determined and measured for all the larvae captured. Totals of 2508 larvae from water tanks, 828 from brooks, and 1203 from fountains were measured. All the specimens were released immediately at their place of capture. Statistical analyses were performed with the packages SPSS 8.0 and STATISTICA 5.5. Mann-Whitney U-tests were performed to test differences in means of post-metamorphic (GSD>42) SVL measurements across reproduction categories.

RESULTS

The calling period of males of *A. dickhilleni* extended from March to August during the study. Empty egg sacs were detected in the water from May to September. A proportion of the tadpole population overwinters. We have not observed an obvious relationship between hatching date and probability of overwintering. Overwintering larvae were found throughout the year (except in fountains, see below). Metamorphic juveniles were found from August to October. We did not observe reproductive activity during the autumn and winter periods.

A large proportion of the total number of tadpoles were at Gosner's stage of development (herein referred to as GSD) 26 (Fig. 1). In fountains, the proportion of tadpoles at GSD 26 gradually decreases after the peak of maximum reproductive activity. However, in water tanks and fountains there are several additional peaks of high proportion of tadpoles at GSD 26 throughout the year.

SVL descriptive statistics for each GSD across breeding sites are shown in Table 2. Larvae from brooks metamorphosed at a larger size than larvae from water tanks or fountains (Mann-Whitney test, GSD's 42 to 46 pooled, water tanks *vs.* brooks, U=649.5, P<0.001; water tanks *vs.* fountains, U=2438.5, P=0.939; brooks *vs.* fountains, U=500, P<0.001). In addition, overwintering larvae were present only in water tanks and brooks, and not in the fountains. The results for the SVL comparisons among breeding site types of stages 26-41 are shown in Table 3. There were no significant differences
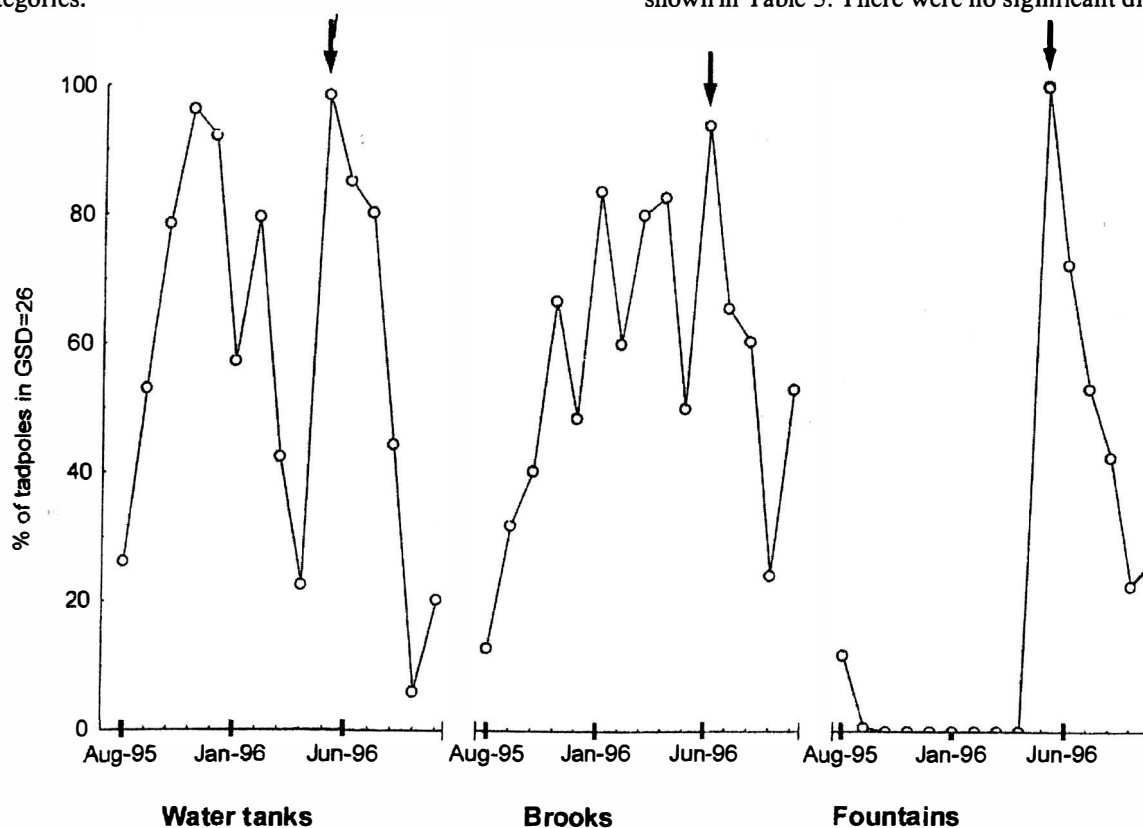

FIG. 1. Percentage of tadpoles at GSD 26 throughout the year in each of the types of breeding sites. 100% = total number of tadpoles sampled in each month. Arrows indicate the period of maximum reproductive activity. In water tanks and brooks, tadpoles in early stages of development are present throughout the year, whereas in fountains they are only present during the breeding season.

TABLE 2. Descriptive statistics for snout-vent length (SVL) measurements of larvae of *A. dickhilleni* at different stages of development (GSD, Gosner, 1960) in three different categories of reproduction. *N* = sample size.

GSD	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
FOUNTAINS																					
Mean	9.31	13.13	14.86	16.10	17.22	17.11	16.38	16.92	17.34	16.90	17.68	17.92	18.91	19.18	20.80	18.04	18.28	17.57	18.35	18.00	18.50
SD	2.46	2.25	2.22	2.45	2.35	2.39	1.72	1.74	1.88	1.83	1.49	1.57	1.88	1.99	1.30	2.11	1.93	2.48	2.37	2.00	0.70
Range	13.5	9.5	10	13	11	13	7	9	9	7	5	7	6	6	3	7	6	12	8	4	1
N	501	137	114	64	43	51	22	57	46	20	16	25	12	11	5	25	14	21	14	3	2
WATER TANKS																					
Mean	12.03	16.70	17.12	16.75	17.74	18.00	17.00	17.87	18.63	18.18	18.69	19.42	20.97	20.19	20.47	18.97	17.38	17.61	18.19	18.2	19.50
SD	4.80	3.83	3.64	2.46	2.76	2.23	1.89	1.65	2.38	1.80	2.03	2.19	1.74	1.32	1.80	1.87	2.19	1.90	1.50	1.10	0.70
Range	21	17	14	13	11	13	9	7	11	6	9	9	6	4	7	8	7	7	5	4	1
N	1646	190	112	70	67	59	20	27	22	29	41	33	36	21	19	24	22	27	21	17	2
BROOKS																					
Mean	13.33	14.24	17.00	17.10	19.06	18.78	20.00	20.41	22.21	21.97	20.78	20.95	20.50	19.75	19.60	18.00	19.71	20.20	22.14	21.00	
SD	4.40	3.50	4.26	3.14	2.56	3.07	3.09	3.08	2.86	3.92	1.53	1.70	1.88	1.42	0.84	2.82	1.49	1.43	3.99	1.58	
Range	18	17	18	9	10	13	8	12	10	18	6	6	6	4	3	4	5	6	18	5	
N	449	60	54	10	38	21	6	17	14	18	23	24	12	12	10	2	7	20	14	17	

in size at each GSD for stages 29, 31, 39, 40 and 41. Stages 26 and 37 were the most variable between sites. In most cases, post-hoc comparisons showed that the significant differences detected were related to a larger size at brooks with respect to water tanks and fountains (Table 3).

In order to evaluate the influence of overwintering larvae on the analyses – and considering that in fountains (where no such tadpoles were detected during the surveys) the maximum SVL recorded was 17 mm – we arbitrarily considered tadpoles $\geq$18 mm at GSD 26 to be overwintering larvae in water tanks and brooks, and excluded them from the analyses. Differences between types of breeding sites were again significant at that stage (ANOVA: F=24.6, P<0.0001), but in this case the differences were related to significantly smaller tadpoles at GSD 26 in water tanks with respect to brooks and fountains, according to post-hoc comparisons.

We also analysed the relationship between size and differentiation across breeding site types. Mean SVL of *A. dickhilleni* larvae are correlated with GSD in all categories (all correlations significant at P<0.01; Spearman's r_s= 0.842 in fountains *vs*. 0.636 and 0.531 for brooks and water tanks, respectively). In brooks and water tanks correlation coefficients were lower than in fountains, probably as a result of the coexistence of newly hatched larvae with overwintering larvae. When these values are excluded from the analyses, corrected correlation coefficients increase to 0.770 and 0.791 in water tanks and brooks, respectively, values that are very close to those obtained for fountains.

DISCUSSION

We found differences in larval phenology and growth history that were related to the type of breeding site used by males of *A. dickhilleni* for the release of their larvae. It has to be stressed, however, that we have not analysed

possible effects of other factors (for example, water quality or density-related crowding effects) that might also partly explain the observed differences in larval growth between breeding site categories. Experiments focused on these interactions should be of help in further testing our hypotheses.

Overwintering larvae were found in water tanks and brooks, but not in fountains. At these sites, low water level was associated with higher predation pressures and a risk of total freezing during cold months in the Sierra Tejeda (Antúnez *et al.*, 1982). Thus, the possibility of surviving winter in fountains may be low, due to the existence of a temporal limit marked by freezing. This might impose restrictions upon individual developmental trajectories, as will be discussed below. Brooks and water tanks do not freeze completely, facilitating tadpole overwintering. Water tanks are cleaned periodically (usually once or twice a year), but sometimes they are not completely emptied during the process, allowing the presence of larvae all along the year.

Variance in developmental traits was detected in the ranges of SVL associated with each GSD. Earlier stages (especially GSD 26, Fig. 2) show significant differences when compared to other stages: their associated ranges of SVL are much larger. We observed that most of the overwintering individuals are at early stages of development. Crespo (1982) noted that earlier stages of development in *A. cisternasii* and *A. obstetricans boscai* represent more than 50% of the time spent at the larval stage. He pointed out that at low temperatures, *Alytes* larvae can stay several months without appreciable morphological development: growth is faster than differentiation at lower temperatures, and vice versa at higher temperatures.

These differences in SVL ranges across GSD's are the consequence of the coexistence of newly-hatched in-

TABLE 3. Results of ANOVAs comparing size at each GSD among different breeding site types. Post-hoc comparisons were based on Tukey's HSD tests for unequal sample sizes (Spjotvol/Stoline test).

| GSD	F	P	Post-hoc comparisons significant
26	110.41	<0.01	All significant
27	48.70	<0.001	Water tanks vs brooks and fountains
28	15.35	<0.001	Brooks vs. water tanks and fountains
29	1.45	N.S.	-
30	5.39	<0.01	Brooks vs. fountains
31	3.88	N.S.	-
32	7.79	<0.01	Brooks vs. water tanks and fountains
33	19.90	<0.001	Brooks vs. water tanks and fountains
34	26.05	<0.001	Brooks vs. water tanks and fountains
35	20.25	<0.001	Brooks vs. water tanks and fountains
36	15.86	<0.001	Brooks vs. water tanks and fountains
37	15.89	<0.001	All significant
38	5.86	<0.01	Water tanks vs. fountains
39	1.56	N.S.	-
40	1.43	N.S.	-
41	1.37	N.S.	-
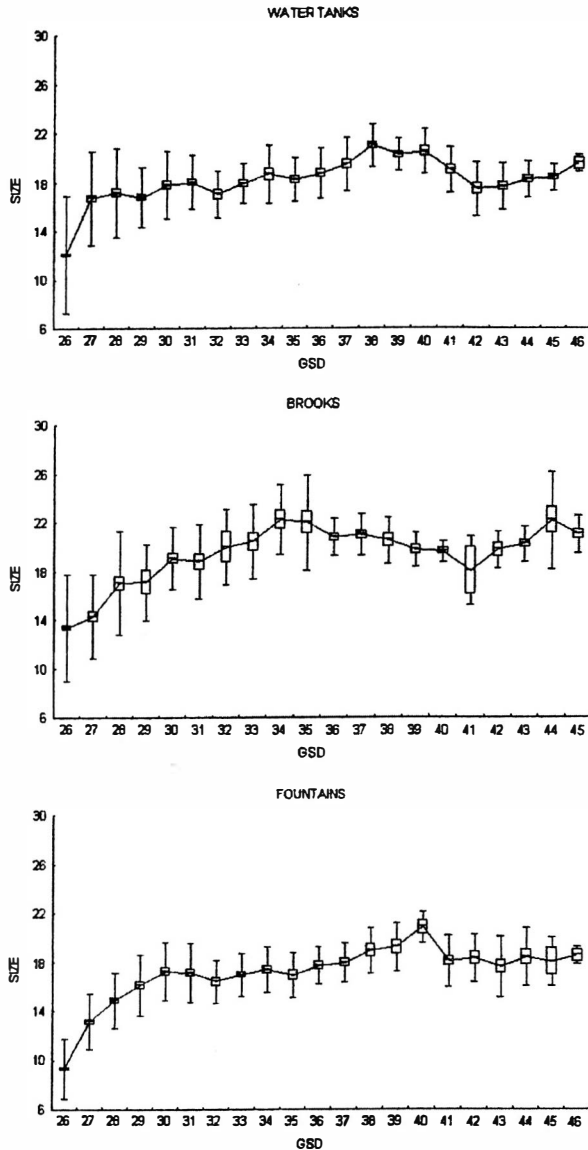
FIG. 2. Mean (line), standard error (box) and standard deviation (vertical bars) of SVL (size) of tadpoles at each stage of development (GSD) in the different types of breeding site.

dividuals and overwintering larvae, both in early stages of development. Apparently, growth and differentiation is decoupled in earlier stages of development such that in some individuals differentiation ceases during winter, while growing still proceeds for several months. Differences in size range associated with each GSD are not expressed equally in all breeding types. In fountains, SVL ranges are of the same magnitude across GSD's, including earlier stages (26-27).

Developmental constraints might limit the possible responses of *A. dickhilleni* larvae to different stability and predictability conditions in the bodies of water in which they grow. In the hylid frogs *Hyla cinerea* and *H. gratiosa,* timing of metamorphosis appears not to be equally plastic at each stage of development, but after a particular threshold is reached, developmental rate appears to be unalterable (Leips & Travis, 1994). In *A. dickhilleni,* our data suggest that earlier stages of development are characterized by a higher plasticity. We

hypothesize that at these plastic stages there would be two possible developmental trajectories: a "fast" one characterized by a linear relationship between size (SVL) and GSD, and a "slow" trajectory where most of the growing takes place during an extended period of time in earlier stages of development, thus the relationship between size and GSD being non-linear. As development progresses past the earliest stages, the plasticity for growing without differentiation would be gradually minimized and the linear ("fast") trajectory would prevail until metamorphosis.

Selection of either strategy might be mediated by differential behavioural responses of developing larvae. In brooks and water tanks tadpoles can select their individual positions along a temperature gradient, since these are established between shallow waters directly exposed to sun radiation and deeper waters where temperatures remain colder. The relation of growth and developmental rates is temperature-dependent (Smith-Gill & Berven, 1979). Thus, it is possible that larvae developing in shallow waters of brook margins or in superficial waters of water tanks, where temperature is higher throughout the summer, could display higher activity and maintain higher metabolic rates than larvae developing in colder waters. In situations where resources are not expected to be limiting, models of resource allocation predict that in early stages these resources will be allocated primarily for development, and after the developmental trajectory is fixed, for growth (Leips & Travis, 1994). Thus, growth and development would be highly correlated, adjusting to the "fast" strategy. The constantly low water level of fountains would prevent the establishment of temperature gradients, and thus only the "fast " strategy would be possible. The differences in the percentage of tadpoles at GSD 26 throughout the year appears to be related to differences in the presence of overwintering individuals in the different breeding sites. At fountains, where no over-wintering individuals were found, the gradual decrease in the percentage of tadpoles at GSD 26 can be explained by the progressive differentiation of tadpoles into more advanced stages over time (Fig. 2). On the other hand, larvae spending most of their time at the bottom of deep pools in brooks, for example, where temperatures are expected to be relatively lower, would also show lower metabolic rates, with the result of delayed development, thus producing deviations from linearity in the growth-development relationship, following a "slow" strategy. Differentiation would even cease in larvae in early stages while they are still growing (though at a slow rate) during the overwintering period. Thus, the additional peaks of high relative numbers of tadpoles at GSD 26 in water tanks and brooks might represent the persistence of tadpoles at early stages of development through the winter period. The steepest declines in the percentage of larvae at GSD 26 in water tanks when compared with brooks, might be related to the periodic cleaning of water tanks, which usually takes place during the summer.

We have shown that variability exists in larval traits across different reproduction sites and across stages of development. Is this variability a by-product of other evolutionary processes or is it the result of selection on developmental traits? If so, what selective advantages would have this variability in a context of variable and often unpredictable environments? This is an important point because costs of maintaining variability in life-history traits must be overcome by its associated benefits (De Witt *et al.*, 1998). The coexistence of two different developmental strategies in populations of a single species may confer upon them higher probabilities of local persistence by allowing a better exploitation of reproductive habitats (Esteban *et al.* 1999). This would be especially important when the availability of reproduction sites is limited, as is the case in south-eastern Spain. *A. dickhilleni* populations are able to reproduce mainly in permanent waters (the usual condition in the genus *Alytes*), but also in temporary ones, such as fountains and some brooks. In these sites, developmental time is shorter than in permanent ponds, which imposes long-term costs in terms of fitness derived from lower sizes at metamorphosis (see, for example, Leips *et al.*, 2000). However, colonization of these sites allows *A. dickhilleni* to maximize the number of ponds in which it breeds, an important issue since sites available for reproduction are a limiting resource in most of its range. Breeding also in permanent waters allows them to prolong the larval period and metamorphose at larger sizes, as observed in brooks. Thus, variability in growth patterns could be related to a better exploitation of heterogeneous habitats by *A. dickhilleni* populations by increasing the range of potential reproductive sites.

ACKNOWLEDGEMENTS

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THE EFFECTS OF HABITAT FRAGMENTATION ON AMPHIBIAN SPECIES RICHNESS IN THE FLOODPLAIN OF THE MIDDLE PARANA RIVER, ARGENTINA

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Key words: amphibian assemblage, cluster analysis, frog diversity

The Middle Paraná river includes a complex system of islands interspersed with riparian woodland and gallery forest, making this an important area for the herpetofauna of Argentina (Bridarolli & di Tada, 1994). However, the Middle Paraná river floodplain is being increasingly fragmented by human activity to the point where existing amphibian populations may be dependent on marginal or altered wetlands for their survival (Peltzer & Lajmanovich, 2001). In this context, some authors have emphasized that amphibians may be vulnerable to habitat fragmentation (e.g. Blaustein *et al.*, 1994; Lavilla, 2001) because of their complex life history patterns and population dynamics (Delis *et al.*, 1996). Moreover, the dispersal patterns of amphibians are likely to be influenced by habitat quality and the distance between suitable patches of breeding habitats (Dodd & Cade, 1998).

In this study, we compare anuran species richness between ponds located in remnants of riparian woodlands representing a range of habitat variables and patch parameters in the Middle Parana river floodplain. The study area is situated in the western zone of the Entre Ríos Province (31° 44'S – 60° 31'W). Vegetation communities are dominated by subtropical elements in contrast to the surrounding temperate spiny vegetation (Burkart *et al.*, 1999). Climatically, this area has an average annual rainfall below 1000 mm and a mean annual temperature of 18°C.

Landsat images and serial aerial photographs (first order to fifth order at a scale of 1:50 000, Brigada Aérea Argentina at Paraná City of Entre Ríos Province) were used to select eight ponds: one located within a pristine riparian woodland (PSM Natural Protected Reserve "Parque General San Martín" - 6940 m²) and seven located in suburban riparian woodlands that differed in degrees of disturbance (VU: Villa Urquiza 1500 m²,

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BG: Bajada Grande - 1300 m², TH: Tomphson - 10 m², PC: Parque Costero - 400 m², PU: Parque Urquiza - 4741 m², AN: Acceso Norte - 2100 m², REG: Regionales - 375 m²).

Field survey. We surveyed the eight ponds for anurans between 1 January 1999 and 31 March 2000. Each month we conducted four or five field trips of about 1-6 hr duration, depending on the pond size and the time spent on collection and identification. We conducted nocturnal site searches to sample adult anurans, beginning shortly after sunset (1900 hr) and extending until midnight. This technique is a combination of the visual encounter surveys of Crump & Scott (1994) and audio strip transects of Zimmerman (1994). A fine-meshed net was used to take samples at different times of the day, but always in daylight. The captured adults and larvae were identified, photographed and then released. Unidentifiable specimens (larval or adult) were fixed in 10% formalin and deposited in the Natural Sciences Museum "Florentino Ameghino" of Santa Fe Province (Argentina) and in the Instituto Nacional de Limnología for later identification.

We recorded nine habitat variables: pH (with Lovibond), air temperature (with a standard thermometer) and maximum depth (with meter). Turbidity was estimated by eye on an ordinal scale of 0-5 (Marnell, 1998), where 0 was crystal clear and 5 was so turbid that light would not penetrate a 5 cm sample jar. Estación Tecnológica Agropecuaria-INTA (located in Paraná City, Entre Ríos Province) provided data on relative humidity and the total monthly volume of precipitation. The noise level was estimated on an ordinal scale, where 0 was an absence of human-generated noise and 3 was a high level of noise (a site near a road, beach or urban development). Aquatic macrophytes and shore vegetation were identified and the vegetation richness for each site was gauged on a qualitative scale ranging from 1 to 5 (Coneza Fernandez Vitora, 1997), where 1 was a monospecific vegetation and 5 was very diverse vegetation. The level of disturbance to the aquatic and surrounding terrestrial habitats was gauged on an arbitrary scale of 0-5 (Pavignano, 1988), where 0 was a natural site without alterations, and 5 was a site completely altered by floods, humans (roads, agriculture, deforestation, excavation, and intentional fire) or farm animals. Moreover, we determined three patch parameters in accordance with the theory of MacArthur & Wilson (1967): pond area (m²), distance (in km) to the pristine riparian woodland, and time since the remnant was isolated (years). We used two levels of distance (1-20 km and >20 km) and two levels of isolation (1-10 years and >11 years). Serial aerial photographs (scales 1:100 000; 1:50 000, and 1:25 000) provided by Brigada Aérea Argentina at Paraná City of Entre Ríos Province were used to estimate time since isolation.

Data analysis. We calculated the similarity between survey sites in terms of the composition of anuran assemblages using the Jaccard similarity measure (Magurran, 1987). The similarity values were used to

construct a site by site similarity matrix for the eight survey sites. We clustered the sites on the similarity of their anuran assemblages using the Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method (Sneath & Sokal, 1973).

We calculated Bravais-Pearson correlation coefficients (Sokal & Rohlf, 1979) to relate species richness to the habitat variables at each site. We also used a multiple regression to relate species richness to pond area, time since isolation, and distance to the pristine riparian woodland. All analyses were performed using the statistical programme Statgraphic version 1.11 (1994).

A total of 20 anuran species belonging to five families was detected in ponds located in remnants of riparian woodland of the Middle Parana river floodplain (Table 1). Three species (*Pseudopaludicola falcipes, Physalaemus riograndensis* and *Physalaemus albonotatus*) were exclusively recorded in the pond within the pristine riparian woodland. Anuran species found on both disturbed and pristine sites were *Bufo fernandezae, B. arenarum, B. paracnemis, Leptodactylus ocellatus, L. chaquensis, L. mystacinus, L. latinasus, L. gracilis, L. elenae, Physalaemus biligonigerus, Hyla nana, H. pulchella, Scinax nasicus, S. squalirostris* and *Elachistocleis bicolor*. The most common species observed in both habitats was *B. arenarum*, recorded in 87.5% of the total ponds sampled, following by *L. latinasus* (75%).

Cluster analysis of the eight surveyed sites based on the similarity of the composition of their anuran assemblages produced two groups (Fig. 1). PSM, P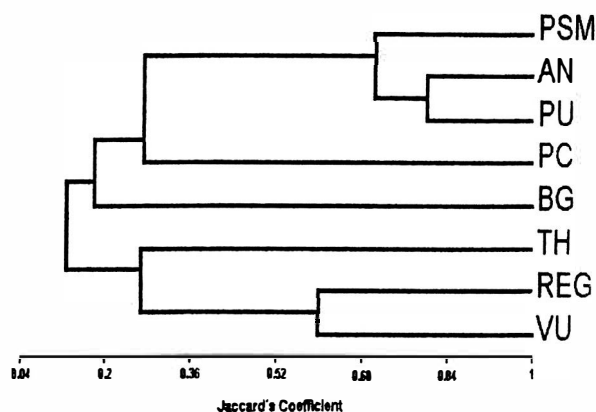U and AN had a close association, while VU and REG formed another distinct group. PC, BG and TH had a distant association with the other two groups.

FIG. 1. Cluster analysis of the eight survey sites, examining the similarity of their anuran richness.

We found six of the nine habitat variables to be significantly correlated with anuran species richness at the study sites: air temperature (r=0.83, df=7, P<0.01), relative humidity (r=0.75, df=7, P<0.01), the logarithm of the water turbidity (r=-0.80, df=7, P<0.01), vegetal richness (r=0.73, df=7, P<0,05), levels of disturbance (r=-0.92, df=7, P<0.01), and noise presence (r= -0.73, df=7, P<0.05). No relationships were found between species richness and water depth, pH or the logarithm of total monthly volume of precipitation.

The combination of pond area, time since isolation and distance to pristine woodland provided the best fit to the variation in species richness (R^2=0.98, F=79.7, df=7, P<0.001). t-tests for the individual explanatory

TABLE 1. Anuran species richness of ponds in riparian woodland of the Middle Parana River. VU, Villa Urquiza; BG, Bajada Grande; TH, Tomphson; PC, Parque Costero; PU, Parque Urquiza; AN, Acceso Norte; REG, Regionales; and PSM, Parque General San Martín. (+) indicates that the species was present and (-) that it was not detected.

Species	Disturbed							Pristine
	VU	BG	TH	PC	PU	AN	REG	PSM
Bufo fernandezae	-	-	-	-	+	+	-	+
Bufo arenarum	+	+	+	-	+	+	+	+
Bufo paracnemis	+	-	-	-	-	-	-	+
Leptodactylus ocellatus	+	-	-	-	+	+	+	+
Leptodactylus chaquensis	-	-	-	-	+	-	-	+
Leptodactylus mystacinus	+	-	-	-	+	+	+	+
Leptodactylus latinasus	+	-	-	+	+	+	+	+
Leptodactylus gracilis	-	-	-	-	-	+	-	+
Leptodactylus elenae	-	-	-	+	+	+	-	+
Physalaemus biligonigerus	-	-	-	+	+	+	-	+
Physalaemus riograndensis	-	-	-	-	-	-	-	+
Physalaemus albonotatus	-	-	-	-	-	-	-	+
Pseudopaludicola falcipes	-	-	-	-	-	-	-	+
Lysapsus limellus	-	+	-	-	-	-	-	-
Hyla nana	-	+	-	+	+	+	-	+
Hyla pulchella	-	-	-	-	+	+	-	+
Scinax nasicus	-	+	-	-	+	+	-	+
Scinax acuminatus	-	-	-	-	+	-	-	-
Scinax squalirostris	-	-	-	-	+	+	-	+
Elachistocleis bicolor	-	-	-	-	+	+	-	+
Species Richness	5	4	1	4	14	13	4	18

variables in the combined model indicated that both pond area and time since isolation contributed significantly to the regression (t_{area}=3.99, df=7, P<0.05; t_{time}=4.38, df=7, P<0.01).

We found anuran richness to be positively related to air temperature, relative humidity and vegetation richness but negatively related to water turbidity, level of noise, and disturbance. The relationship between temperature, relative humidity, and anuran richness is consistent with previous observations in tropical habitats in the Brazilian Amazon (Tocher et al., 1997). In this context, the reproductive activity of anurans in tropical and subtropical areas depends on the combination of temperature and rainfall (Duellman & Trueb, 1986). However, the calling activity and presence of the species in ponds located in remnants of riparian woodland were not correlated with the total monthly volume of precipitation. This may be because South America experienced a severe drought throughout the period of the study associated with what many consider the strongest El Niño-Southern Oscillation (post-ENSO) event of the last century (Hammond & ter Steege, 1998; McPhaden, 1999). The scarce records of the common frogs Scinax acuminatus and Lysapsus limellus and the complete absence of Hyla raniceps, a species previously recorded for this area (pers. obs.), may be due to the dry weather and a consequent lack of suitable breeding sites.

Anuran populations may be concentrated in areas of more appropriate habitat surrounding urban development, thereby increasing relative richness in these areas. In our study, the riparian woodlands of the Middle Paraná river floodplain with no – or only low levels of – disturbance were found to be even more significant as refuges from urban and agricultural development. Roads, deforestation, excavation, agricultural activities and livestock grazing are increasing the isolation of remnants of riparian woodland and contributing to habitat fragmentation. The potential effects of agricultural pesticides and fertilizers on amphibian growth, development and suitable breeding sites should also be considered (Tyler & Williams, 1996; Bishop et al., 1999). Scinax nasicus, Bufo arenarum and Physalaemus biligonigerus larvae have been found to be sensitive to cypermethrin and paraquat (Lajmanovich et al., 1998; Izaguirre et al., 2000).

We found a significant correlation between anuran richness and plant species richness at the ponds surveyed. Aquatic vegetation and shore vegetation in the riparian areas are important for anurans, providing them structural heterogeneity, moisture, shelter, calling sites, and places to attach their eggs (Stumpel & van der Voet, 1998).

Two of the patch measurements, pond area and time since isolation, contributed significantly to the regression model of anuran richness. The positive relationship between anuran richness and pond area may be due to a habitat-island effect (MacArthur & Wilson, 1967), with the lowest richness being predicted for the smallest patch (10 m^2). Some studies have investigated the ef-

fects of patch area on amphibians (e.g. Zimmerman & Bierregard, 1986; Hecnar & M'Closkey, 1997) and found it to be the most important variable in determining amphibian species richness. Although we have not considered the anuran richness of the matrix, it is possible that anurans may disperse through the riparian corridors, transitional lands adjacent to streams, oxbows and gallery forest. This may explain the absence of a statistically significant relationship between anuran richness and distance to pristine woodland.

Our results indicate that altered remnants of riparian woodland in the Middle Parana river floodplain with low temperature and relative humidity, monospecific vegetation, turbid water and proximity to urban development or roads have few anuran species. Although these results are preliminary, it is evident that habitat modification and fragmentation of the riparian woodland of the Middle Parana river by human activities have resulted in a decrease in anuran richness. On the other hand, the limited information available on the biology and dispersal capability of the amphibians of the study area (Lajmanovich, 1997; 2000; Peltzer & Lajmanovich, 1999; 2000; 2002) makes it difficult to asses how they might be impacted by habitat alteration.

We conclude that the likelihood of maintaining biological diversity in the riparian woodlands of the Middle Parana river floodplain will be increased by protection of the relatively scarce remnants, and minimization of further fragmentation and disturbance of the landscape.
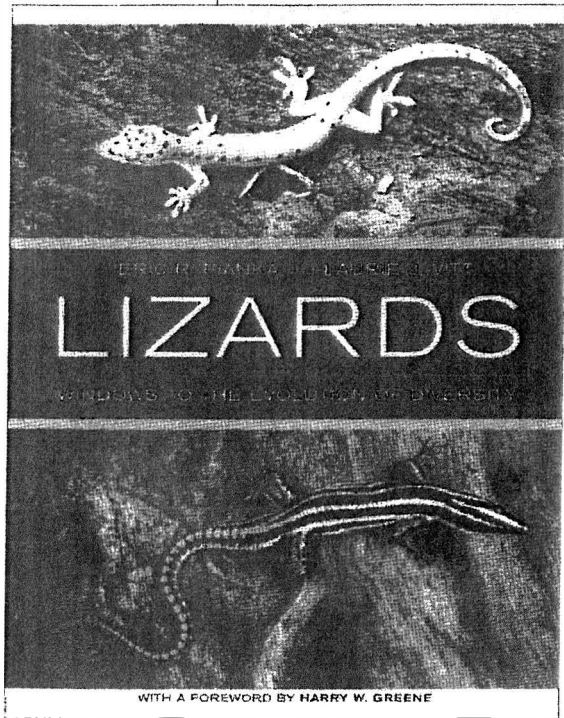
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THE HERPETOLOGICAL JOURNAL

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