



Huge but moderately long-lived: age structure in the mountain chicken, *Leptodactylus fallax*, from Montserrat, West Indies

Fabio Maria Guarino¹, Gerardo Garcia² & Franco Andreone³

¹Dipartimento di Biologia, Università di Napoli Federico II, Via Cintia, 21, I-80126, Napoli, Italy

²North of England Zoological Society, Chester Zoo, Upton-by-Chester, CH2 1LH, United Kingdom

³Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, I-10123 Torino, Italy

We applied skeletochronological methodology to assess the age and growth in one of the largest living anurans, the mountain chicken *Leptodactylus fallax*. We analysed bone cross-sections obtained from wild animals found dead after a chytrid outbreak in Montserrat in 2009, and from captive individuals which are part of a breeding program at Jersey Zoo. Lines of arrested growth (LAGs) were visible in both groups. Individuals reared in captivity reached an older age than their wild counterparts. At the same age, captive males and females were larger than wild individuals. A literature screening of age and snout-vent length (SVL) for 46 species ascribed to seven families (Bufonidae, Dicroglossidae, Leptodactylidae, Mantellidae, Microhylidae, Ranidae and Rhacophoridae) showed that the largest species were also the most long-lived. With a maximum documented SVL of 280 mm and a maximum longevity of 9 years, *L. fallax* reached a higher body size as predicted by age, representing a case of gigantism probably associated with adaptation to an insular environment.

Key words: islands, *Leptodactylus*, longevity, Montserrat, mountain chicken, skeletochronology, tropical anurans

INTRODUCTION

The mountain chicken (*Leptodactylus fallax*) is one of the largest frogs in the world, currently living only on the islands of Montserrat and Dominica, West Indies (Kaiser, 1994; Breuil, 2004). Because of its large body size, it has been long utilised as a food item, and this drove it to extinction in some islands of the archipelago (Fa et al., 2010). Besides unsustainable over-harvesting, several other threats affect its survivorship: introduction of alien predators such as rats and cats (Kairo et al., 2003), deforestation and habitat loss and an outbreak of chytridiomycosis in Montserrat in 2009 (Fisher et al., 2009; García et al., 2009). Due to these threats and its narrow distribution, *L. fallax* was assessed as Critically Endangered on the IUCN Red List of Threatened Species (Fa et al., 2010).

The present paper provides information about the age of mountain chickens, and the reliability of skeletochronology to assess the species' longevity. We also investigated whether the large body size of *L. fallax* is a consequence of exceptional longevity or rapid growth. The tendency to increase body size on islands is shared by reptiles and birds whereas the reverse is the case for mammals (Raia et al., 2010; Raia & Meiri, 2011).

Insular gigantism is generally related to geographical and ecological isolation, lack of predators and reduced interspecific competition for the same trophic niche (Petren & Case, 1997; Lomolino, 2005; Meiri et al., 2010); such explanatory hypotheses for insular gigantism or dwarfism are however difficult to disentangle (Keogh et al., 2005). To this end, we also compared the longevity and the unusually large size of *L. fallax* with those of other tropical frog species.

MATERIALS AND METHODS

Study area

The island of Montserrat has three volcanic massifs along its north-south axis: Silver Hills (maximum altitude 403 m a.s.l.), Centre Hills (740 m) and Soufriere Hills (915 m). Mountainous terrain covers 102 km², of which only 33 km² are habitable. The Centre Hills are covered with relatively continuous rainforest and characterised by deep, radial drainage valleys of roughly circular shape (Laurance et al., 2002). *Leptodactylus fallax* occurs in the Centre Hills, from sea level to the once forested peaks of the Soufriere Hills at 915 m (Martin et al., 2007). The Centre Hills forest is relatively intact and of high conservation importance (Young, 2008).

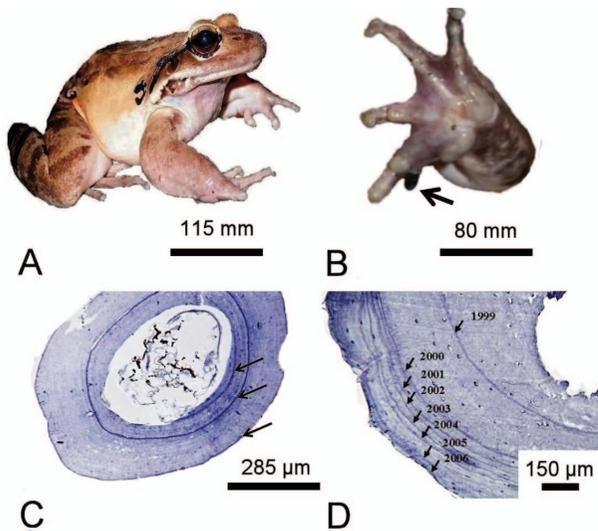


Fig. 1. The studied species *Leptodactylus fallax*: (A) adult male, (B) the peculiar hand spur present in sexually mature males, as indicated by a black arrow, (C) a bone section of a wild female (SVL=130 mm) with three LAGs shown by a black arrow, and (D) a bone section of a captive female (SVL=158 mm), of 9 years, with 8 LAGs highlighted by a black arrow and the corresponding year of reference (the 9th LAG is not visible due to a process of erosion, while the first visible LAG is double).

Sampling and body measurements

We sampled 43 individuals (13 males and 30 females). The corpses of 22 individuals (4 males and 18 females) were collected in March 2009 in their natural environment after a chytrid outbreak (García et al., 2009) from a locality known as Pelican Ghaut (about 100 m a.s.l.; coordinates: 16°45.0’N, 62°10.0’W). Twenty-one individuals (9 males and 12 females) were further collected in Montserrat in July and November 1999 as juveniles or small-sized adults and housed in captivity until they died. Due to their size these individuals were considered as being at least one

year old. They were housed in pairs (one male and one female) in indoor enclosures (200x200 cm) under natural temperatures (20–25°C from April to September, and 24–30°C during the remaining months) and a photoperiod of 1412 to 1212 hours. This group was part of a captive breeding program which begun at Jersey Zoo in 1999 (Fa et al., 2010).

The snout-vent length (SVL) of frogs found dead in the field was measured to the nearest 0.1 mm, and the sex was determined through analysis of external secondary sexual characters. Males had a smaller body size, hypertrophied forearms, pointed spurs at the base of the thumb and a dark throat. The pointed spur is considered to indicate sexual maturity in males, and is used for amplexus and combat with other males (Kaiser 1994; Khan & KU Herpetology Class, 2004; Silva et al., 2005). Since we could not unequivocally determine the smallest size of mature females, we established a threshold of 110 mm SVL, corresponding to the smallest male found with evident spurs. Every smaller individual was ascribed to the unsexed category of “juveniles”. This size is approximate when applied to both the sexes, since we assume that mature females have a larger minimum SVL.

Skeletochronological analysis

The 3rd toe of the right foot of individuals found dead in the wild or in captivity was clipped and stored in 70% ethanol. A standard skeletochronology protocol was followed to assess individual age through the count of the lines of arrested growth (LAGs, Guarino et al., 1998, 2010; Andreone & Guarino, 2003). Phalanges were decalcified in 5% nitric acid for 2–3 hours, and washed in running tap water overnight. Cryostat cross-sections at diaphyseal level, 12 µm thick, were stained for 30 minutes in Mayer’s acid hemalum and mounted in aqueous resin.

For each toe, at least ten sections were observed using a Motic BA400 light microscope equipped with a

Table 1. Snout-vent length (SVL) (precision of 0.1 mm) and number of lines of arrested growth (LAG) of *Leptodactylus fallax* from Montserrat. Asterisks indicate the significance of Mann-Whitney test: * $p < 0.05$, ** $p < 0.01$.

Wild individuals		Captive individuals		U Mann-Whitney test Wild vs. Captive adults	
SVL	LAGs	SVL	LAGs	SVL	LAGs
Juveniles					
-	-	83.7±11.6	1.1±0.3		
-	-	(65.0–99.0)	(1–2)		
		10	10		
Males					
154.0±25.0	4.25±1.26	156.5±22.9	6.0±0.89	11.5	3
(140.0–190.0)	(3–6)	(128–190)	(5–7)		
4	4	6	6		
Females					
168.8±35.4	4.78±1.10	144.2±11.7	8.0±1.22	17*	2.5**
(130.0–280.0)	(3–7)	(132–158)	(6–9)		
18	18	5	5		
U Mann-Whitney test					
Males vs. Females					
23	25	9	3*		

Table 2. Tropical anuran species, study area, altitude, and maximum age in both sexes assessed by skeletochronology, LAGs=lines of arrested growth. M=males, F=females. n.s.=not specified. Asterisks indicate low-mid altitudes species considered for the regression analysis.

Family	Species and used acronyms	Area	Altitude (m.asl)	Maximum snout-vent length (mm)	Maximum longevity	Reference	
Bufonidae	<i>Bufo bankorensis</i> * (Bb)	S Taiwan	10–460	82.7	5	Lin & Hou, 2002	
	<i>Duttaphrynus melanostictus</i> * (Dm)	SE India	45	78.0 (M), 104.0 (F)	5 (M), 11 (F)	Nayak et al., 2007	
	<i>Atelopus chiriquiensis</i> (Ac)	W Panama	2200	33.3 (M), 46.1 (F)	4	Lindquist et al., 2012	
	<i>Atelopus peruvensis</i> (Ap)	N Peru	2800–4200	45.5 (M), 44.7 (F)	6	Lindquist et al., 2012	
	<i>Atelopus lozanoi</i> (Al)	Colombia	3000–3300	29.3 (M), 46.2 (F)	4	Lindquist et al., 2012	
	<i>Atelopus</i> sp. (<i>nusagandii</i>) * (Asp)	Panama	600	30.3 (M)	3	Lindquist et al., 2012	
	<i>Atelopus zeteki</i> * (Az)	C Panama	1000	37.6 (M), 47.0 (F)	3	Lindquist et al., 2012	
	<i>Euphyllactis cyanophlyctis</i> * (Ec)	SE India	n.s.	65.0 (M), 8.1 (F)	5 (M), 7 (F)	Kulkarni & Pancharatna, 1996; Pancharatna et al., 2000	
	<i>Fejervarya limnocharis</i> (Fl)	W Java, Indonesia	220, 478, 800	53.0	1	Kusrini & Alford, 2006	
	<i>Fejervarya cancrivora</i> (Fc)	W Java, Indonesia		56.7	1	Kusrini & Alford, 2006	
Leptodactylidae	<i>Limnonectes macrotodon</i> * (Lm)	W Java, Indonesia	n.s.	105.2	5	Kusrini & Alford, 2006	
	<i>Hoplobatrachus tigerinus</i> * (Ht)	SE India	n.s.	170.0	6	Kumbar & Pancharatna, 2001	
	<i>Limnonectes limnocharis</i> * (Ll)	SE India	n.s.	45.0	4	Pancharatna & Kumbar, 2005	
	<i>Leptodactylus fallax</i> * (Lf)	Montserrat, Lesser Antilles	0–915	190.0 (M), 280.0 (F)	7 (M), 9 (F)	Pancharatna & Deshpande, 2003	
						This paper	
	Mantellidae	<i>Boophis tsilomaro</i> * (Bt)	NW Madagascar	170	84.6 (M)	11 (M)	Andreone et al., 2002
		<i>Boophis williamsi</i> (Bw)	CE Madagascar	2000–2314	40.0 (M), 42.6 (F)	6 (M), 5 (F)	Andreone et al. 2014
		<i>Mantella aurantiaca</i> , <i>M. baroni</i> , <i>M. bernhardi</i> , <i>M. cowani</i> , <i>M. crocea</i> , <i>M. expectata</i> , <i>M. laevigata</i> , <i>M. nigricans</i> , <i>M. pulchra</i> , <i>M. viridis</i> * (Msp)	Several areas of Madagascar	120–1400	31.2 (M), 32.5 (F)	4 (M), 4 (F)	Guarino et al., 2008b, 2010; Jovanovic & Vences., 2010; Andreone et al., 2011
		<i>Mantidactylus pauliani</i> (Mp)	CE Madagascar	2000–2314	35.8 (M), 35.3 (F)	8 (M), 8 (F)	Andreone et al. in press
		<i>Microhyla heymansi</i> * (Mh)	Taiwan	10, 460	30.4	2	Lin & Hou, 2002
<i>M. ornata</i> * (Mo)		Taiwan	10, 460	25.2	3 (M), 4 (F)	Kumbar & Pancharatna, 2001	
Ranidae	<i>M. steinegeri</i> * (Ms)	Taiwan	10, 460	29.0	4		
	<i>Dyscophus antongilii</i> * (Da)	NE Madagascar	10, 486	75.1 (M), 98.9 (F)	7 (M), 11 (F)	Tessa et al., 2007	
	<i>Dyscophus guineti</i> * (Dg)	E Madagascar	670, 690	90.7 (M), 112.4 (F)	6 (M), 7 (F)	Tessa et al., 2011	
	<i>Scaphiophryne gottlebei</i> * (Sg)	S Madagascar	510, 1268	33.0 (M), 44.2 (F)	2 (M), 2 (F)	Guarino et al., 2010	
	<i>Hylarana latouchii</i> * (Hl)	S Taiwan	30–150	50.4	1	Lin & Hou, 2002	
	<i>Hylarana nigrovittata</i> * (Hn)	E Thailand		67.9 (M), 53.9 (F)	9 (M), 6 (F)	Khonsue et al., 2000	
Rhacophoridae	<i>Polypedates maculatus</i> * (Pm)	SE India	n.s.	60.0	3	Kumbar & Pancharatna, 2001	
	<i>Buergeria japonica</i> (Bj)	S Taiwan	n.s.	35.5	0	Lin & Hou, 2002	
	<i>Polypedates megalophthalmus</i> (Pe)	S Taiwan	n.s.	59.6	0	Lin & Hou, 2002	

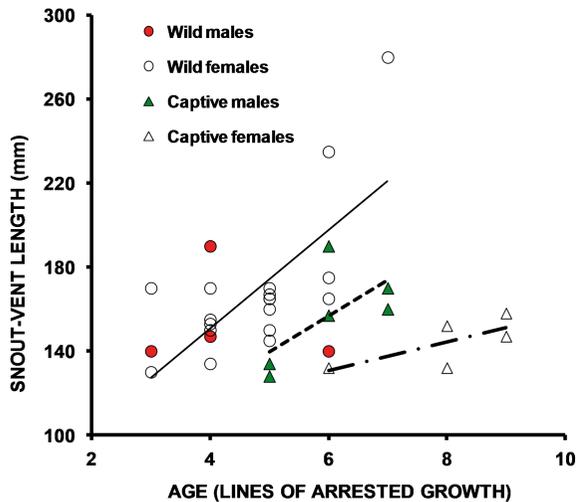


Fig. 2. Plots between longevity and SVL in four samples of *Leptodactylus fallax*, from captivity and from the wild. Regression lines are provided for all the samples except for wild males.

Nikon Coolpix 5000 digital color video camera. Age was assessed independently by two observers who counted the number of periosteal LAGs. In case of discrepancies between the counted numbers of LAGs, the sections were read again until a final consensus was reached. When two sets of counts were different the lower count was taken into account.

Following Driscoll (1999) and Miaud et al. (1999), we selected sections with the smallest marrow cavity in order to avoid or minimize the effects of endosteal resorption, a potential cause of destruction for the innermost LAGs (Castanet et al., 1993). In addition, we compared the perimeters of the outer margin of the smallest individuals with the “reversal line” (the resorption line between periosteal and endosteal bone) of the large-sized individuals in order to assess the possible loss of one or more inner LAGs via the “back calculation” method (Guarino et al., 2008a).

Literature review for skeletochronological studies on tropical anurans

For comparison of body size and age of other species, we examined the scientific literature for skeletochronological studies on tropical anurans (living between 23°27 North and South). For the genus *Mantella*, 10 species were pooled to get an overall “*Mantella* spp.” category due to overall similar life history traits and longevities across species (Andreone et al., 2011).

Since *L. fallax* lives at an altitude of up to 915 m a.s.l. (the highest peak of Montserrat) we selected only the species found at low-mid altitudes (from 0 to 1500 m) to minimise the effect of altitude on longevity since species living at high altitudes are in general more long-lived than species from low altitudes (Miaud et al., 1999; Morrison et al., 2004). We excluded data for *Fejervarya limnocharis* and *F. cancrivora* for which longevity values of only one year are reported (Kusrini & Alford, 2006), as well as those of *Buergeria japonica* and *Polypedates megacephalum* for which LAGs were not observed (Lin

& Hu, 2002). For statistical analysis we used the software packages STATISTICA v.7.0 and PAST v.0.94 (PAST, 2002).

RESULTS

Bone histology and mark interpretation

All individuals showed evident lines of arrested growth (LAGs), each counted as one year (Fig. 1). The first (innermost) two LAGs were often faintly stained and associated with an array of false lines. Lines from the third LAG onward were sharper and more evident. In specimens with more than three LAGs, the peripheral LAGs were usually closer and often confluent with each other, thus determining the splitting of one broad and dark LAG into numerous LAGs.

Complete erosion of 1–2 perimedullar LAGs due to endosteal resorption was observed in about 19% of captive frogs and inferred by osteometrical analysis in about 27% of wild frogs. Complete resorption of perimedullar LAGs was observed only in the oldest individuals. Thus, simple counting of visible LAGs may underestimate the actual age, especially in older animals, but osteometrical analysis allowed for corrections for the absence of LAGs due to bone resorption and remodeling.

Body size, longevity and growth

Individuals ranged from 74 to 280 mm in SVL (Table 1). The maximum SVL in males was 190 mm in both the wild and captive groups. In females, the maximum SVL was 154 mm in the wild group and 280 mm in the captive group. Females were larger than males in wild individuals, although the difference was not significant ($p=0.29$). Captive females were significantly smaller than wild individuals ($p=0.04$).

Maximum estimated age was 6 and 7 years in captive and wild males, and 9 and 7 years in captive and wild females, respectively. The differences between captive males and captive females ($p=0.03$) as well as between wild and captive females ($p=0.001$) were significant (Table 2). In the captive group, 11 individuals (52.4%) showed LAG numbers corresponding to the time of housing; six individuals (28.6%) with small size (SVL<110 mm) showed one more LAG than the years in captivity;

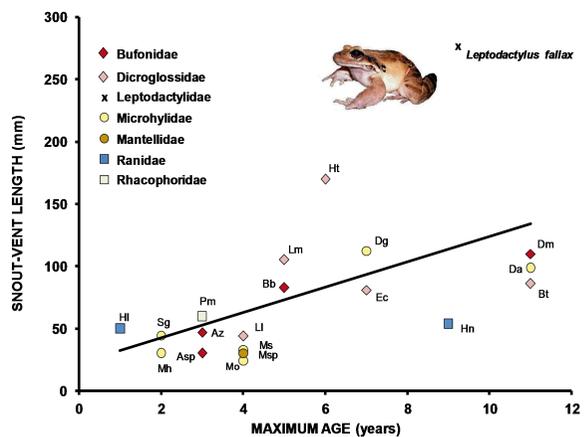


Fig. 3. Plots and regression lines in seven anuran families from tropical areas, including *Leptodactylus fallax*. Used acronyms are reported in Table 2.

two individuals (9.5%, both SVL 132 mm) had one LAG fewer, and three individuals (14.3%, SVL 147–170 mm) had two LAGs fewer than their years in captivity.

Based upon osteometrical analysis we estimated a loss of one LAG in one male and five females of the wild group. LAG number and age were correlated in both sexes of the captive group (males, $r=0.92$, degrees of freedom (df)=7, $p<0.001$; females, $r=0.95$, df=10, $p<0.001$) and in females of the wild group ($r=0.70$, df=16, $p<0.01$, Fig. 3). Wild males were not considered due to their small number ($n=4$).

Single factor ANCOVA (with sex as the factor and age as the covariate) showed that, at the same age, captive males and females differed significantly for SVL ($F=38.56$, df=1, 18, $p<0.01$). Due to the low number of wild males we did not perform the ANCOVA between wild males and wild females. A comparison of females caught in the wild and captive females showed that there was a significant difference in the slope of the regression (Fig. 2). In particular, ANCOVA on slopes indicated that wild females were larger than captive females of a same age ($F=19.68$, df=1, 27, $p<0.01$).

Longevity and body size in tropical anurans

The list of tropical anuran amphibians studied with skeletochronology is reported in Table 2. The data include seven families (Bufonidae, Dicroglossidae, Leptodactylidae, Mantellidae, Microhylidae, Ranidae and Rhacophoridae) and 46 species, ranging from 25.2 (*Microhyla ornata*) to 280 mm SVL (*Leptodactylus fallax*), and from one year (*Hylarana latouchii*) to 11 years (*Dyscophus antongili*) of age. Some species (i.e., *Buergeria japonica* and *Polypedates megacephalum* and one population of *D. antongili*) did not show any evident LAGs (Lin & Hou, 2002; Tessa et al., 2007). There was a positive correlation between body size and age ($r=0.52$, $p<0.05$).

DISCUSSION

Applicability of skeletochronology on tropical anurans

Our study revealed that LAGs were present in both wild and captive *L. fallax*. In wild individuals, LAGs are likely formed during the dry season (between December and June) when food is less abundant and temperatures are lower. This is in agreement with observations of other tropical and subtropical species (Khonsue et al., 2000; Andreone et al., 2002; Kumbar & Pancharatna, 2002; Tessa et al., 2007; Liao & Lu, 2010a, b; Liao et al., 2011).

LAGs were also observed in captive individuals, for which the alternation of warm/cold periods was maintained in captivity. For captive individuals we found partial agreement between the number of LAGs and the years of housing. This was probably due to faintly stained lines and/or endosteal resorption. As a general rule, false lines were mostly observed internally to the 2nd LAG, suggesting that repeated arrests of growth occurred more frequently during the first years of life. Moreover, we assume that the estimated age at capture was sometimes incorrect because early growth in *L. fallax* can be very rapid. In captive frogs, the overall consistency of

age estimates with actual age supports the assumption that LAGs are formed annually.

LAGs, body size and growth in the mountain chicken

The documented age in wild *L. fallax* was lower than the age achieved by individuals kept in captivity, in agreement with previous data from other species. As an example, the small-sized *Mantella* species from Madagascar are reported to live 3–4 years in nature (Andreone et al., 2011), but may reach 10 years of age in captivity (de Magalhaes & Costa, 2009). It is also worth noting that captive females are smaller than wild animals. Alimantation and general condition in captivity might have been suboptimal for females and may have caused a reduction in growth rates. Captive males also lived less long than females in captivity, which has previously been observed in other amphibians (*Amietophrynus pardalis*, Cherry et al., 1992; *Pelophylax perezii*, Esteban et al., 1996).

The sex ratio of wild animals collected after the chytrid outbreak appeared female biased, and only four males were collected (García et al., 2009). This contrasts with observations from August–October 2009, when the sex-ratio was almost equal (G.M. Rosa, pers. comm.). Females collected in the wild and females in captivity showed different growth rates, indicating that growth is affected by environmental conditions. Individuals reared in captivity likely experienced a more regular temperature and humidity, and constant food supply. This parallels the presence of false lines, particularly in young individuals (as observed previously for example with *Pelobates varaldi*, Guarino et al., 2011).

Our findings confirm that *Leptodactylus* species lack sexual size dimorphism (Silva et al., 2005); in our sample, the largest individual with a SVL of 280 mm (which also represents the known species' size record) was a female. A comparison of the distance between single LAGs suggested that sexual maturity is reached after 2–3 years with a SVL of around 110 mm, a size which corresponds to about 50% of the maximum body length. In captivity, males attain a larger size than females at given age. Female amphibians are generally larger than males (Halliday & Verrell, 1986), except for species with territorial behaviour and male-male competition (Shine, 1979). In *L. fallax*, male-male competition is a well-known behavioural strategy, evidenced by pointed spurs on the forearms of males (as is also the case for other *Leptodactylus* species, Prado & Haddad, 2003).

Longevity and body size in tropical anurans

LAG deposition in *L. fallax* confirms the validity of skeletochronology as a method to assess age for tropical anurans. Without seasonality, however, LAG deposition becomes less evident or non-existent (Lin & Hou, 2002; Kusriani & Alford, 2006; Tessa et al., 2007; Segev et al., 2012). The maximum longevity of *L. fallax* recorded was 9 years, although a maximum age of 15 years would be predicted considering body size and known age of other species (Fig. 3). *Dyscophus antongili* and *Boophis tsilomaro* from Madagascar are the tropical anurans with the highest longevity, reaching a maximum length of 99

mm and 43 mm, respectively (Andreone et al. 2002; Tessa et al., 2007). This suggests that the large body size in the mountain chicken is due to more rapid and continuous growth than in the other anurans considered. Apart from potential effects of phylogeny we assume that most of the constraints which affect longevity in anurans are ecological. We suggest that the large body size observed in *L. fallax* is associated with island gigantism (Lomolino, 2005). *Leptodactylus fallax* is the only native frog living in Montserrat, where autochthonous predators are absent or scarce. Both factors may have produced a relaxed selective pressure and the utilisation of a wide range of prey, leading to large body size. The mountain chicken has a broad trophic niche, including insects, millipedes, crustaceans and other vertebrates such as other frogs, snakes and small mammals (Daltry, 2002). We associate the late attainment of sexual maturity in *L. fallax* with the large size reached by the species, assuming that a protracted juvenile growth pattern allows for attainment of a larger size. This trend has also been documented for two giant insular lizards, *Chioninia coctei* from Cape Verde (Andreone & Guarino, 2003) and in *Gallotia simonyi* from Canarias (Castanet & Baez, 1991). This rule, summarised by Shine & Charnov (1992) is also here invoked for an anuran (*L. fallax*).

ACKNOWLEDGEMENTS

The authors are grateful to G. Gray, J. Lopez, C. Black Fenton, L. Martin, J. (Scriber) Daley, J. Greenaway, P. (Mapie) Murrain, S.-L. Adams, A. Fernandez, G. M. Rosa, K. Bradfield, M. Goetz, and the Durrell Wildlife Conservation Trust for support and participation in the fieldwork. S. Walker, J. Wilkinson and P. Tierney helped with the English language.

REFERENCES

- Andreone, F., Crottini, A., Rabemananjara, F.C.E., Randrianirina, J.E., et al. (2014). Age structure, population estimate and *Bd*-status of two Critically Endangered frogs from the Ankaratra Massif (Madagascar), *Boophis williamsi* and *Mantidactylus pauliani* (Amphibia: Mantellidae). In Capula M. & Corti C. (Editors). *Scripta Herpetologica. Studies on Amphibians and Reptiles in honour of Benedetto Lanza*. Monografie della Societas Herpetologica Italica – III, Edizioni Belvedere, “le scienze” 19, pp. 17–29.
- Andreone, F., Giacoma, C., Guarino, F.M., Mercurio, V. & Tessa, G. (2011). Age profile in nine *Mantella* poison frogs from Madagascar, as revealed by skeletochronological analyses. *Alytes* 27, 73–84.
- Andreone, F. & Guarino, F.M. (2003). Giant and long-lived? Age structure in *Macroscoincus coctei*, an extinct skink from Cape Verde. *Amphibia-Reptilia* 24, 459–470.
- Andreone, F., Vences, M., Guarino, F.M., Glaw, F. & Randrianirina, J.E. (2002). Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frogs. *Journal of Zoology* 257, 425–438.
- Breuil, M. (2004). *Amphibiens et reptiles des Antilles*. PLB Editions, Abymes.
- Castanet, J. & Baez, M. (1991). Adaptation and evolution in *Gallotia* lizards from the Canary Islands: age, growth, maturity and longevity. *Amphibia-Reptilia* 12, 81–102.
- Castanet, J., Francillon-Vieillot, H., Meunier, F.J. & de Ricqlès, A. (1993). Bone and individual aging. Pp. 245–283 in B.K. Hall (Ed.). *Bone growth*. CRC Press, Boca Raton.
- Cherry, M.I. & Francillon, M.H. (1992). Body size, age and reproduction in the leopard toad, *Bufo pardalis*. *Journal of Zoology London* 228, 41–50.
- Daltry, J.C. (2002). Mountain chicken monitoring manual. Fauna and Flora International, Cambridge, and the Forestry and Wildlife Division, Dominica.
- de Magalhaes, J.P. & Costa, J. (2009). A database of vertebrate longevity records and their relation to other life-history traits. *Journal of Evolutionary Biology* 22, 1770–1774.
- Driscoll, D.A. (1999). Skeletochronological assessment of age structure and population stability for two threatened frog species. *Australian Journal of Ecology* 24, 182–189.
- Esteban, M., Paris, M.G., & Castanet, J. (1996). Use of bone histology in estimating the age of frogs (*Rana perezi*) from a warm temperate climate area. *Canadian Journal of Zoology* 74, 1914–1921.
- Fa, J., Hedges, B., Ibéné, B., Breuil, M., et al. (2010). *Leptodactylus fallax*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. Available from: <<http://www.iucnredlist.org>>. Accessed: 31 December 2013.
- Fisher, M.C., Garner, T.W.J., & Walker, S.F. (2009). Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* 63, 291–310.
- García, G., Lopez, J., Fa, J. E. & Gray, G.A.L. (2009). Chytrid fungus strikes mountain chickens in Montserrat. *Oryx* 43, 323–328
- Guarino, F.M., Andreone, F. & Angelini, F. (1998). Growth and longevity by skeletochronological analysis in *Mantidactylus microtypanum*, a rain-forest anuran from southern Madagascar. *Copeia* 1998, 194–198.
- Guarino, F.M., Di Già, I. & Sindaco, R. (2008a). Age structure by skeletochronology in a declining population of *Rana temporaria* from northern Italy. *Acta Zoologica of the Academy of Sciences of Hungary* 54, 99–112.
- Guarino, F.M., Tessa, G., Sacco, M. & Andreone, F. (2008b). Short life span of two charismatic Mantella species: age-structure in the critically endangered *M. cowani* and in the syntopic *M. baroni*. In Andreone F. (Editor). *A Conservation Strategy for the Amphibians of Madagascar – Monografie XLV*, Museo Regionale di Scienze Naturali di Torino, pp. 265–276.
- Guarino, F.M., Tessa, G., Mercurio, V. & Andreone, F. (2010). Rapid sexual maturity and short life span in the blue-legged frog and the rainbow frog from the arid Isalo Massif, southern-central Madagascar. *Zoology* 113, 378–384.
- Guarino, F.M., de Pous, P., Crottini, A., Mezzasalma, M. & Andreone, F. (2011). Age structure and growth in a population of *Pelobates varaldii* (Anura, Pelobatidae) from northwestern Morocco. *Amphibia-Reptilia* 32, 550–556.
- Halliday, T.R. & Verrell, P.A. (1986). Review: sexual selection and body size in amphibians. *Herpetological Journal* 1, 86–92.
- Jovanovic, O. & Vences, M. (2010). Skeletochronological analysis of age structure in populations of four species of Malagasy poisonous frogs, genus *Mantella*. *Amphibia-Reptilia* 31, 553–557.
- Kairo M., Ali, B., Cheesman, O., Haysom K. & Murphy, S. (2003).

- Invasive species threats in the Caribbean Region. CABI, UK.
- Kaiser, H. (1994). *Leptodactylus fallax*. *Catalogue of American Amphibians and Reptiles*, 583, 1–3.
- Keogh, J.S., Scott I.A. & Hayes, C. (2005). Rapid and repeated origin of insular gigantism and dwarfism in Australian tiger snakes. *Evolution*, 59, 226–233.
- Khan, Z. & KU Herpetology Class. (2004). *Leptodactylus fallax*. Mountain Chicken; Giant Ditch Frog; Crapaud. AmphibiaWeb: Information on amphibian biology and conservation. [web application]. 2012. Berkeley, California: AmphibiaWeb. Available from: <<http://amphibiaweb.org/>>. Accessed: January 31, 2013.
- Khonsue, W., Matsui, M. & Misawa, Y. (2000). Age determination by skeletochronology of *Rana nigrovittata*, a frog from tropical forest of Thailand. *Zoological Science* 17, 253–257.
- Kulkarni, J.T. & Pancharatna, K. (1996). Age related changes in ovarian follicular kinetics in the Indian skipper frog *Rana cyanophlyctis* (Schn). *Journal of Bioscience* 21, 699–710.
- Kumbar, S.M. & Pancharatna, K. (2001). Occurrence of growth marks in the cross sections of phalanges and long bones of limbs in tropical anurans. *Herpetological Review* 32, 165–167.
- Kumbar, S.M. & Pancharatna, K. (2002). Annual growth layers in the phalanges of the Indian skipper frog *Rana cyanophlyctis* (Schn.). *Copeia* 2002, 870–872.
- Kusrini, M.D. & Alford, R.A. (2006). The application of skeletochronology to estimate ages of three species of frogs in West Java, Indonesia. *Herpetological Review* 37, 423–425.
- Laurance, W.F., Lovejoy, T.E., Vasconcelos, H.L., Bruna, E.M., et al. (2002). Ecosystem decay of Amazonian forest fragments: a 22 year investigation. *Conservation Biology* 16, 605–618.
- Liao, W.B. & Lu, X. (2010a). Age and growth of a subtropical high-elevation torrent frog, *Amolops mantzorum*, in Western China. *Journal of Herpetology* 44, 172–176.
- Liao, W.B. & Lu, X. (2010b). Age structure and body size of the Chuanxi treefrog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China). *Zoologische Anzeiger* 248, 255–263.
- Liao, W.B., Lu, X. Shen, Y.W. & Hu, J.C. (2011). Age structure and body size of two populations of the rice frog *Rana limnocharis* from different altitudes. *Italian Journal of Zoology* 78, 215–221.
- Lin, Y.L. & Hou, P.L. (2002). Applicability of skeletochronology to the anurans from a subtropical rainforest of southern Taiwan. *Acta Zoologica Taiwanica* 13, 21–30.
- Lindquist, E., Redmer, M. & Brantner, E. (2012). Annular bone growth in phalanges of five Neotropical harlequin frogs (Anura: Bufonidae: *Atelopus*). *Phyllomedusa* 11, 117–124.
- Lomolino, M.V. (2005). Body size evolution in insular vertebrates: generality of the island rule. *Journal of Biogeography* 32, 1683–1699.
- Martin, L., Morton, M.N., Hilton, G.M., Young, R.P., et al. (2007). *A species action plan for the Montserrat mountain chicken* *Leptodactylus fallax*. Department of Environment, Montserrat.
- Meik, J.M., Lawing, A.M. & Pires-da Silva, A. (2010). Body Size Evolution in Insular Speckled Rattlesnakes (Viperidae: *Crotalus mitchellii*). *PLoS ONE* 5, e9524.
- Miaud, C., Guyétant, R. & Elmer, J. (1999). Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology* 249, 61–73.
- Morrison, C., Hero, J.M. Browning, J. (2004). Altitudinal variation in the age at maturity, longevity, and reproductive lifespan of anurans in subtropical Queensland. *Herpetologica* 60, 34–44.
- Nayak, S., Mahapatra, P.K., Mohanty, R.K. & Dutta, S.K. (2007). Age determination by skeletochronology in the Common Indian Toad *Bufo melanostictus* Schneider, 1799 (Anura: Bufonidae). *Herpetozoa* 19, 111–119.
- Pancharatna, K. & Deshpande, S.A. (2003). Skeletochronological data on age, body size and mass in the Indian Cricket Frog *Limnonectes limnocharis* (Boie, 1835). *Herpetozoa* 16, 41–50.
- Pancharatna, K. & Kumbar, S.M. (2005). Estimation of age and longevity of the Indian bullfrog *Hoplobatrachus tigerinus* (Daudin, 1802): a skeletochronological study. (Anura, Ranidae). *Herpetozoa* 18, 147–153.
- Pancharatna, K., Sapna, C. & Kumbar, S.M. (2000). Phalangeal growth marks in relation to testis development in the frog, *Rana cyanophlyctis*. *Amphibia Reptilia* 21, 371–379.
- PAST. (2002). Data analysis package, version 3.X. Øyvind Hammer, DAT Harper, Oslo.
- Petren, K. & Case, T.J. (1997). A phylogenetic analysis of body size evolution and biogeography in chuckwallas (*Sauromalus*) and other Iguanines. *Evolution* 51, 206–219.
- Prado, C.P. & Haddad, C.F.B. (2003). Testes size in leptodactylid frogs and occurrence of multimale spawning in the genus *Leptodactylus* in Brazil. *Journal of Herpetology* 37, 354–362.
- Raia, P., Guarino, F.M., Turano, M., Polese, G., Rippa, et al. (2010). The blue lizard spandrel and the island syndrome. *BMC Evolutionary Biology* 10, 289.
- Raia, P. & Meiri, S. (2011). The tempo and mode of evolution: body sizes of island mammals. *Evolution* 65, 1927–1934.
- Segev, O., Andreone, F., Pala, R., Tessa, G. & Vences, M. (2012). Reproductive phenology of the tomato frog, *Dyscophus antongilli*, in an urban pond of Madagascar's East coast. *Acta Herpetologica* 7, 331–340.
- Shine, R. (1979). Sexual selection and sexual dimorphism in the Amphibia. *Copeia* 1979, 297–306.
- Shine, R. & Charnov, E.L. (1992). Patterns of survival, growth, and maturation in snakes and lizards. *American Naturalist* 139, 1257–1269.
- Silva, W.R., Giaretta, A.A. & Facure, K.G. (2005). On the natural history of the South American pepper frog, *Leptodactylus labyrinthicus* (Spix, 1824) (Anura: Leptodactylidae). *Journal of Natural History* 39, 555–566.
- Tessa, G., Guarino, F.M., Giacomini, C., Mattioli, F. & Andreone, F. (2007). Longevity and body size in three populations of *Dyscophus antongillii* (Anura, Microhylidae), the tomato frog from north-eastern Madagascar. *Acta Herpetologica* 2, 139–146.
- Tessa, G., Guarino, F.M., Randrianirina, J.E. & Andreone, F. (2011). The age structure in the false tomato frog *Dyscophus guineti* from eastern Madagascar, compared to the closely related *D. antongillii* (Anura, Microhylidae). *African Journal of Herpetology* 60, 84–88.
- Young, R.P. (ed.) (2008). *A biodiversity assessment of the Centre Hills, Montserrat*. Durrell Conservation Monograph 1. Durrell Wildlife Conservation Trust, Jersey.

Accepted: 20 January 2014