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FRONT COVER: O'Shaughnessy's chameleon, Calumma oshaughnessyi (L. Brady)

MONITORING A BREEDING POPULATION OF CRESTED NEWTS (*TRITURUS CRISTATUS*) IN A HOUSING DEVELOPMENT

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A housing estate was built immediately adjacent to two water bodies utilized by crested newts (*Triturus cristatus*). Conservation management, particularly pond deepening and fish removal, was undertaken during and after development. This paper describes a monitoring study to determine the success of the operation. Counting adults at night in the breeding season indicated that numbers were at least maintained over a ten year period. Larval counts were made in each of the seven years after the houses were constructed, and more than half of the larvae were netted in one year, 1991. In the autumn of 1990 both ponds were totally dry, and success in 1991 may have been associated with absence of fish and low numbers of invertebrate predators. The overall conclusion was that it is possible to conserve crested newts satisfactorily in such a situation.

INTRODUCTION

Many of the world's amphibian species are declining as a result of local or global factors (e.g. Blaustein & Wake, 1995). The crested newt (Triturus cristatus) ranges widely across Europe, but is under threat in many countries (Steward, 1969; Corbett, 1994). It is listed on Appendix II of the Berne Convention and Annexes II and IV of the European Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora. In Britain, it receives protection through listing on Schedule 5 of the Wildlife and Countryside Act, 1981 and on Schedule 2 of the Conservation (Natural Habitats & c.) Regulations, 1994. Important sites may be notified as Sites of Special Scientific Interest under Part II of the Wildlife and Countryside Act. It is again recognized as in need of protection by being on the short list of Annex F in the UK's Biodiversity Action Plan. Special Areas of Conservation (SACs) will be designated throughout the European Union as part of the Natura 2000 series to assist the conservation of this species.

Conservation of the crested newt in Britain was reviewed by Gent & Bray (1994). Amongst the principal threats to British populations of this species is urban development for housing and other purposes (Cooke & Scorgie, 1983; Hilton-Brown & Oldham, 1991). One option, where a development is allowed to take place, is to attempt to conserve the species within the development. As with any novel form of management, there should be monitoring of its success. To date, however, no such event has been monitored over a number of years and the results published. This paper reports the monitoring of a crested newt population before, during and after construction of a housing development, both to determine whether such a solution may be a viable option in similar situations elsewhere and to provide information on monitoring methodology.

SITE AND ITS HISTORY

The site is located at TL 202961 in Stanground, near Peterborough. The history of newts at the site has not been documented, but it is well-known locally as a traditional breeding site and the ponds have existed since at least the last century.

In 1986, the Nature Conservancy Council (NCC, now English Nature) was consulted over a planning proposal by Persimmon Homes to convert horse pasture adjacent to East and West ponds into a housing estate (Fig. 1). The initial night count in 1986 (see below) revealed the presence of crested newts in both ponds and demonstrated the importance of the site. NCC confirmed that the ponds should be retained together with some surrounding land and, in view of the uncertain effects on the hydrology of the site, asked for a tap to be installed by East pond.

The area set aside for the ponds and their surroundings was about 1 ha. As this was considered unlikely to be sufficient to accommodate the newts totally (Cooke, 1985; Oldham & Nicholson, 1986; Oldham, 1994), access to adjacent gardens was facilitated by not erecting solid fences or walls (however, a few such fences have been erected in the intervening years). The Frank Perkins Parkway, which is a dual carriageway, probably represents a barrier to the north-west (e.g. see Oldham, 1994); but access to fragmented, suitable habitat within a few hundred metres to the north, east or south is relatively easy (Fig. I). Crested newts are occasionally seen crushed on the lane to the south of West pond.

Construction of houses began in 1987, after the newt breeding season, and was completed by 1989. House density is about 16/ha over the estate as a whole. Since 1989 the site has been managed as a reserve with newt conservation as its primary aim.



FIG. 1. A sketch map showing the ponds and their surroundings, as they were in 1995. Existing houses and gardens are cross-hatched; houses and gardens in the new development are stippled. Netting locations in East and West ponds are indicated by crosses.

East pond measures roughly 40 m x 20 m, while West pond is up to 80 m long and 10 m wide. North pond lies about 200 m to the north east of East pond (Fig. 1). Although North pond was not directly affected by the housing development, its hydrology has been modified (see later). Some amenity management has been done to its surrounds which extend to about 0.2 ha. The pond measures 30 m x 25 m. No other crested newt breeding site is known within 600 m (unpublished and T. Langton, pers. comm.).

METHODS

Both adult and larval phases of the newts were studied. Counting at night with a torch was used as a means of monitoring the comparative size of the breeding population (Griffiths & Raper, 1994; Langton et al., 1994; Oldham, 1994; Cooke, 1995; Griffiths et al., 1996). Water turbidity and amount of aquatic vegetation were recorded when counts were made. During the pre-development phase, one count was done for East and West ponds in 1986 and two in 1987. Thereafter three or four counts were undertaken during the main part of each breeding season (March-May). The principal objective of conserving newts at the site was to maintain and, if possible, enhance the crested newt population. The smooth newt (Triturus vulgaris) population was of secondary interest but was monitored at the same time. The aim of night counting was to be able to detect long term trends rather than short term variations between years. Regression analysis was performed on annual means to determine whether there was a significant change in newt numbers post-development or over the entire ten year period, 1986-1995. Means were used primarily because there was some

Year	No. of		Crested Newts		Smooth Newts				
	counts	East pond	West pond	Total	East pond	West pond	Total		
Pre-develo	pment								
1986	1	93	9	102	17	1	18		
1987	2	28 <u>+</u> 0	1 <u>+</u> 1	29 <u>+</u> 1	8 <u>+</u> 4	2 <u>+</u> 2	10 <u>+</u> 6		
During dev	velopment								
1988	3	16 <u>+</u> 12	0	16 <u>+</u> 12	3 <u>+</u> 2	1 <u>+</u> 1	4 <u>+</u> 2		
Post-devel	opment								
1989	4	51 <u>+</u> 4	17 <u>+</u> 6	67 <u>+</u> 15	24 <u>+</u> 7	18 <u>+</u> 2	42+8		
1990	3	41 ± 15	10 ± 2	51 ± 16	7 <u>+</u> 2	9 <u>+</u> 2	16 ± 2		
1991	3	93 <u>+</u> 30	13 <u>+</u> 6	106 <u>+</u> 36	41 <u>+</u> 24	18 <u>+</u> 4	59 <u>+</u> 27		
1992	3	62 <u>+</u> 11	12 <u>+</u> 5	74 <u>+</u> 16	18 <u>+</u> 4	27 <u>+</u> 10	45 <u>+</u> 12		
1993	3	102 <u>+</u> 17	20 <u>+</u> 6	123 <u>+</u> 17	68 <u>+</u> 32	57 ± 10	125 <u>+</u> 35		
1994	3	59 <u>+</u> 28	16 <u>+</u> 5	75 <u>+</u> 33	29 <u>+</u> 6	20 <u>+</u> 6	49 <u>+</u> 12		
1995	3	44 <u>+</u> 23	10 <u>+</u> 4	55 <u>+</u> 26	23 <u>+</u> 17	10 <u>+</u> 4	33 <u>+</u> 19		

TABLE 1. Night counts of newts in East and West ponds, 1986-1995. Values given are mean counts±SE.

Year	night	counts	
	Crested newts	Smooth newts	
1986	17	5	
1987	16	6	
1988	20, 2	5, 8	
1989	31	13	
1990	30	8	
1991	39	20	
1992	29	17	
1993	19	25	
1994	0	1	
1995	4	15	

TABLE 2. Night counts of newts in North pond, 1986-1995

variation in the number of counts per year. North pond was also counted at night once (or twice) each spring for comparison, being considered in 1986 to be far less vulnerable to impacts from the development.

Each year post-development, East and West ponds were netted for larvae in a standard fashion: for each pond, the sumps and the western ends (Fig. 1) were each swept with a pond net for five minutes on a total of four dates during July and August. Whenever possible, visits were on fine, dry days. Catches should give a comparative indication of numbers during the late larval period (see Cooke & Cooke, 1993; Cooke, 1995). There was no systematic netting of North pond.

RESULTS AND OBSERVATIONS

Night counts are given in Tables 1 and 2 with larval catches in Table 3. In general, all three ponds had conditions suitable for torch counting (Griffiths *et al.*, 1996). During the night counts, <1% of crested newts recorded were believed to be immatures, based on small size (<10 cm total length); in view of this low number of immatures, they are not differentiated in Tables 1 and 2.

Pre-development counts were variable, but the local importance of the site was confirmed by the combined

count for East and West ponds in 1986 of 102 crested newts (Table 1). The main drain for the housing estate was installed in April 1988 and by June this appeared to have had a marked effect on the hydrology of the site, with the water table being 40-50 cm below the bed of West pond. Both ponds were therefore re-excavated with a mechanical digger in December 1988. Although reasonable numbers of adult crested and smooth newts returned to breed in 1989 and 1990 (Table 1), production of metamorphs failed in 1990 (Table 3). This failure was associated with an increase in three-spined sticklebacks (Gasterosteus aculeatus) caught in East pond (a mean \pm SE of 3 \pm 1 were netted in 1989, with 26 \pm 2 in 1990) and total desiccation of West pond. (A means of piping the water to west pond was not devised until 1991). In October 1990, East pond was pumped dry and the fish removed. No fish have been seen or caught there since then.

Larval catches in 1991 were high for smooth newts and exceptional for crested newts; indeed, more than 50% of the entire larval catch for crested newts, 1989-1995, occurred in 1991 (Table 3). The summers of 1991-1993 were marked by stable, high water conditions in East and West ponds, with the tap being used when necessary. Larval production for crested newts declined in both ponds over this period (Table 3). In contrast, numbers of smooth newt larvae remained high throughout the period. Numbers of larvae of both species were very low in 1995; the tap was little used and West pond was reduced to a few puddles by late summer and East pond was confined to the sump.

Regression analysis was done on mean night count data for both species in East pond, West pond and both ponds over the periods 1986-1995 and 1989-1995. Of the 12 analyses, 11 gave positive slopes, although there were no significant relationships between newt numbers and time. Thus populations in the ponds in the reserve were at least maintained. Numbers of the two species were correlated in East pond (1985-1995, $r_s = 0.77$, P<0.01; 1989-1995, $r_s = 0.79$, P<0.05) and West pond (1985-1995, $r_s = 0.88$, P<0.01; 1989-1995, $r_s = 0.71$, P = 0.05).

TABLE 3. Catches of larval newts in East and West ponds, July-August, 1989-1995. * indicates a total of 1-3 larvae caught during the year. Values represent mean catch±SE.

no. of	crested newt larvae			smooth newt larvae					
occasions	East pond	West pond	Total	East pond	West pond	Total			
4	16 <u>+</u> 5	13 <u>+</u> 4	29 <u>+</u> 8	3 <u>+</u> 2	4 <u>+</u> 1	6 <u>+</u> 2			
4	0	0	0	<] *	0	<1*			
4	37 <u>+</u> 3	62 <u>+</u> 7	99 <u>+</u> 9	13 <u>+</u> 4	22 <u>+</u> 2	35 <u>+</u> 3			
4	14 <u>+</u> 5	15 <u>+</u> 2	29 <u>+</u> 7	22 <u>+</u> 1	27 <u>+</u> 7	49 <u>+</u> 7			
4	1 <u>+</u> 1	<1*	2 <u>+</u> 1	12 <u>+</u> 4	15 <u>+</u> 6	26 <u>+</u> 8			
4	10 <u>+</u> 3	2 <u>+</u> 2	13 <u>+</u> 3	19 <u>+</u> 9	6 <u>+</u> 5	24 <u>+</u> 15			
4	<1*	0	<1*	<1*	2+2	2+1			
	no. of netting occasions 4 4 4 4 4 4 4 4 4 4	no. of created and the create	$\begin{array}{c} \text{no. of} \\ \text{netting} \\ \hline \\ \text{occasions} \end{array} \begin{array}{c} \text{Crested newt larva} \\ \hline \\ \text{East pond} \end{array} \begin{array}{c} \text{West pond} \\ \hline \\ 4 \\ 4 \\ 0 \\ 0 \\ 4 \\ 37 \pm 3 \\ 4 \\ 14 \pm 5 \\ 15 \pm 2 \\ 4 \\ 14 \pm 5 \\ 15 \pm 2 \\ 4 \\ 1 \pm 1 \\ 4 \\ 10 \pm 3 \\ 2 \pm 2 \\ 4 \\ 4 \\ -1 * \\ 0 \end{array} \begin{array}{c} \text{orbital} \\ \text{orbital} \\$	$\begin{array}{c cccc} \text{no. of} & \text{crested newt larvae} \\ \hline \text{netting} \\ \hline \text{occasions} & \overline{\text{East pond}} & \text{West pond} & \text{Total} \\ \hline \\ 4 & 16\pm5 & 13\pm4 & 29\pm8 \\ 4 & 0 & 0 & 0 \\ 4 & 37\pm3 & 62\pm7 & 99\pm9 \\ 4 & 14\pm5 & 15\pm2 & 29\pm7 \\ 4 & 14\pm5 & 15\pm2 & 29\pm7 \\ 4 & 1\pm1 & <1* & 2\pm1 \\ 4 & 10\pm3 & 2\pm2 & 13\pm3 \\ 4 & <1* & 0 & <1* \\ \end{array}$	$ \begin{array}{c cccc} \text{no. of} & \text{crested newt larvae} & & \text{sm} \\ \hline \text{netting} & & \hline \\ \text{occasions} & \overline{\text{East pond}} & \text{West pond} & \text{Total} & \hline \\ \hline & \text{East pond} \\ \hline \\ 4 & 16\pm5 & 13\pm4 & 29\pm8 & 3\pm2 \\ 4 & 0 & 0 & 0 & <1* \\ 4 & 37\pm3 & 62\pm7 & 99\pm9 & 13\pm4 \\ 4 & 14\pm5 & 15\pm2 & 29\pm7 & 22\pm1 \\ 4 & 1\pm1 & <1^* & 2\pm1 & 12\pm4 \\ 4 & 10\pm3 & 2\pm2 & 13\pm3 & 19\pm9 \\ 4 & <1^* & 0 & <1^* & <1^* \\ \end{array} $	$\begin{array}{c cccc} \text{no. of} & \text{crested newt larvae} & \text{smooth newt larv} \\ \hline \text{netting} & \hline \text{cast pond} & \text{West pond} & \text{Total} & \hline \text{East pond} & \text{West pond} \\ \hline \\ 4 & 16\pm5 & 13\pm4 & 29\pm8 & 3\pm2 & 4\pm1 \\ 4 & 0 & 0 & 0 & <1* & 0 \\ 4 & 37\pm3 & 62\pm7 & 99\pm9 & 13\pm4 & 22\pm2 \\ 4 & 14\pm5 & 15\pm2 & 29\pm7 & 22\pm1 & 27\pm7 \\ 4 & 1\pm1 & <1* & 2\pm1 & 12\pm4 & 15\pm6 \\ 4 & 10\pm3 & 2\pm2 & 13\pm3 & 19\pm9 & 6\pm5 \\ 4 & <1* & 0 & <1* & <1* & 2\pm2 \\ \hline \end{array}$			

In contrast, in North pond there was a significant decline in crested newts counted at night over the period 1989-1995 (Table 2, slope of line significantly different from zero, $t_s = 3.52$, P<0.05). By the mid 1990s, counts of crested newts in North pond were low following unsympathetic amenity management. In 1993. overhanging branches were removed, thereby reducing shaded, open areas in which breeding newts were frequently recorded. In 1994, the hydrology of the pond was changed and the water level lowered. Since 1994, the shallow edges have been increasingly invaded by Typha latifolia. North pond was totally dry by August 1995, the first time I had seen it in this condition (i.e. it did not desiccate during the drought of 1991-92).

DISCUSSION

In this exercise, East and West ponds were allowed to remain, but the terrestrial habitat to the north was considerably altered by the construction of the housing estate. The original terrestrial environment was mainly closely-grazed pasture and, apart from use for foraging, was likely to be of relatively little value to newts which prefer structurally diverse habitats (Oldham & Nicholson, 1986; Oldham, 1994). The land in the reserve immediately surrounding the ponds has been left unmanaged except for some seasonal mowing. The newts also have access to gardens, both those of the new estate houses and those of the older houses. The amount of accessible terrestrial habitat of a newtfriendly type (Oldham, 1994) is therefore unlikely to be less than existed in the mid 1980s.

Pond conditions were generally suitable for torch counting, so this convenient and relatively non-intrusive technique was employed throughout. Night counting and larval netting enabled problems to be identified and subsequently corrected. The two principal problems encountered were desiccation in 1988 and fish predation in 1990. The significance of the latter factor in particular would not have been appreciated without focused monitoring.

The success of fish removal from East pond in autumn 1990 might be judged by the good production of crested and smooth newt larvae in the pond in 1991 following failure in 1990. However, poor production in non-desiccation years could not simply be blamed on fish, as crested newt larval numbers declined in East pond in 1992 and 1993 in the absence of fish. Similar changes in larval numbers occurred in West pond 1991 to 1993 following natural desiccation in 1990 (fish have never been recorded in this pond). These changes may have been due to populations of aquatic invertebrate predators recovering after both ponds were dry in 1990. For instance, dragonfly larvae may be important predators, but those species with a long period of larval development will be more affected by pond dessication (Smith, 1983; Arntzen & Teunis, 1993). Although dragonfly larvae were not counted during netting in

East and West ponds, they were noted as being particularly numerous in 1992 and 1993. If predation by invertebrates is serious at a site, then management might aim to allow the ponds to desiccate in autumn. The temptation is always to maintain plenty of water to allow larval development and metamorphosis in all parts of a pond. But the magnitude of larval numbers of crested newts at this site in 1991 indicates that this may not lead to the best results in the longer term. The highest night counts of adults occurred in 1993, perhaps because of high numbers of two-year-old newts returning to breed for the first time (see Francillon-Vieillot et al., 1990; Arntzen & Teunis, 1993; Oldham, 1994). Natural desiccation contributed to, or caused total (or near total), breeding failure in 1990 and 1995. But the benefits of the tap were demonstrated during the national drought which led to desiccation and breeding failure in other local sites in 1991 and 1992 (Cooke, 1995).

One problem with monitoring the success of a venture such as this is that often there are few baseline observations made before development takes place. For this reason, regression analysis of annual mean counts was used to test trends. Dodd & Seigel (1991) reviewed whether documented examples of relocation, repatriation and translocation (RRT) of amphibians and reptiles have succeeded; they contended that most experiments are monitored for an insufficient time to judge their success. Although the Stanground project is not an example of RRT, it nevertheless requires comparable monitoring to determine its success. Crested newts are capable of living for 10 years or more (Oldham & Nicholson, 1986; Francillon-Vieillot et al., 1990), so individuals might have survived from the pre-development period until 1995. However, fluctuations in counts (Table 1) indicated appreciable recruitment of adults in 1991 and 1993 at least. The conclusion to date is that populations of both species of newt have been at least maintained, but it should be appreciated that without detailed monitoring and subsequent corrective management, the exercise may have failed. Monitoring will continue, at least in the short term to check that good reproduction of crested newts is recorded again.

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DETERMINATION OF MINIMUM SAMPLE SIZE TO ESTIMATE DIET DIVERSITY IN ANURAN SPECIES

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Rarefaction analysis was applied to determine the minimum sample size required for any desired degree of accuracy of diet diversity of six anuran species in a particular habitat. This method is suggested for the design of sampling strategies in long-term amphibian studies. In the present study the minimum sample sizes were 28, 36, 32, 13, 24 and 8 for *Rana esculenta*, *R. arvalis*, *Hyla arborea*, *Bombina bombina*, *Pelobates fuscus* and *Bufo bufo*, respectively. The greater the diet diversity of a species, the larger the minimum sample size required, and the smaller the similarity between individuals within species.

INTRODUCTION

Since 1984 we have studied the spatial and temporal variation of food diversity of anuran species in a protected marshland area. One methodological problem that has arisen is the comparison of diet diversity when different numbers of each species are sampled. When using common diversity indices to quantify the degree of diet selectivity of anurans it is clear that sample size (number of individuals) affects the calculated value. To determine the sample size required for any desired degree of accuracy, rarefaction analysis was chosen (Simberloff, 1972; Heck et al., 1975; James & Rathbun, 1981), as it is in no way specific for one type of sampling method when applied to the measurement of diet composition of anurans. From the rarefaction curves we estimated the number of sampled individuals needed to reach at least 95% of the whole-sample diversity.

An additional advantage of applying the required minimum sample size is that the disturbance of anuran communities can be minimized.

METHODS

The study was conducted in Diás Island, located in a protected wetland area of south-west Hungary. Anurans were collected in an alder swamp forest and meadow covering the central part of the island, in 1992 and 1993 (Török & Csörgö, 1992). This island is no larger than 1 ha, and according to our observations, specimens of the studied species use both habitats for feeding. To avoid the influence of seasonal differences in diet, we collected three samples of equal size in spring, summer and autumn. A total of 641 stomach contents of anuran specimens belonging to six species were analysed (Bombina bombina 42% [juveniles]; Pelobates fuscus, Bufo bufo, Hyla arborea 30%; Rana esculenta complex 24% in 1992, 45% in 1993; R. arvalis 49% in 1992, 33% in 1993). To obtain the food, a stomach flush method was used (Fraser, 1976; Legler & Sullivan, 1979; Opatrny, 1980; Griffiths, 1986). A 10 cm long plastic cannula 1 mm in diameter was attached to a 10 ml medical syringe. The cannula was gently inserted through the mouth and into the stomach.

We used 5-50 ml water depending on the size of the frogs or toads.

Prey was preserved in 70% methanol. A total of 9117 prey items were identified to class, order or family level. In the present analysis a total of 38 taxa were represented as the specimens could not all be identified to species level.

DATA ANALYSIS

Several indices have been used to estimate the diet diversity (niche breadth) and diet similarity (niche overlap) of different animal species (Colwell & Futuyma, 1971; Hurlbert, 1978, 1982; Abrams, 1980; Feinsinger *et al.*, 1981; Griffiths, 1986). We used the Shannon-Weaver index to estimate the diversity of the diet (Shannon & Weaver, 1949):

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

where p_i is the proportion of prey category *i* (by number). *S* is the number of categories.

Rarefaction analysis is a method designed to determine how diversity changes with increasing sample size. Subsets of x samples were used, where x varies from one to the actual number of samples (N) used to calculate diversity. If possible, 200 subsets of size xwere randomly chosen and the average diversity estimated. In general, rarefaction curves asymptote when the sample size is large enough to show that the estimation is no longer dependent on sample size. We accepted the sample size which gave at least 95% of the diversity calculated for the total sample size of a species. Two types of function (exponential and hyperbolic) can be applied to compute the minimum sample size. We used the hyperbolic as this function converges to a limit (H_{lim}) , which is the maximal diet diversity for a given species in a given habitat

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H=H_{lim}-a(1/x)
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where x is the number of the anuran specimens and a is the slope.

			1992		1993							
	$H_{\rm lim}$	а	F	Р	n _s	$H_{\sf lim}$	а	F	Р	n _s		
R. esculenta c.	2.65	-3.34	595.1	0.001	22	2.65	-3.70	205.2	0.001	28		
R. arvalis	2.50	-2.48	1169.0	0.001	20	2.59	-4.71	142.9	0.001	36		
H. arborea	/	/	/	/	/	2.35	-3.79	458.6	0.001	32		
B. bombina	/	/	/	/	/	1.81	-1.15	322.1	0.001	13		
P. fuscus	2.19	-1.80	876.1	0.001	16	2.21	-2.67	283.4	0.001	24		
B. bufo	1.48	-0.42	187.8	0.001	6	1.14	-0.46	124.8	0.001	8		

TABLE 1. Slope (a) and asymptote (H_{im}) of hyperbolic curves fitted to data of rarefaction analysis, and the minimum number of anurans (n) required to estimate the 95% limit of diet diversity (ANOVA for goodness of fit).

Species for which more stomachs are required to reach a diversity plateau have higher diet diversity than other species. To quantify this we calculated the similarity between individuals using the proportional similarity index (Renkonen, 1938):

 $S_{15} = \sum \min(p_{11}; p_{21})$

where $p_{1,i}$ is the proportion of prey category *i* in one individual, $p_{2,i}$ in the other individual.

To calculate both diversity and similarity indices we used software by Dolph Schluter (Schluter, 1988) which was available from the author upon request.

RESULTS AND DISCUSSION

Rarefaction analysis was used to produce curves where the different asymptotes reflected the differences in diet diversity among the anuran species (Fig.1). The slopes of the curves increased steeply with increasing asymptotes (Fig. 2). The observed values fitted each curve closely (Table 1).

The most common anurans were the two Rana species in the study area. Both of them usually eat a variety of prey species showing a high diversity (Kovács & Török, 1992; Török & Csörgö, 1992). Similarly to these results, Medvedev (1974), Zimka (1974), Loman (1979) and Löw et al (1990) found high diet diversity in Rana arvalis. In our study the mean number of prey items per stomach ranged between 6.2-12.5 (Table 2). Zimka (1971) found similar values (5.6-7.2) in R. arvalis stomachs. Külhorn (1960) studied the diet of R. esculenta and also showed diverse food composition where the most common prey groups were Coleoptera, Gastropoda, Diptera, Homoptera and Araneidea. This pattern was similar to the present findings, except for Gastropoda and Homoptera, which were underrepresented in the diet of our R. esculenta population. Both Rana species were numerous in the study area which made it possible to collect large data sets for an accurate estimate of the rarefaction curves. For the Rana esculenta complex, 95% of diet diversity can be estimated from 22 and 28 individuals in the two years.



FIG. 1. Hyperbolic curves fitted to data of rarefaction analysis. Vertical lines show the number of anurans needed to estimate the 95% of their diet diversity (RE=Rana esculenta complex, RA=R. arvalis, HA=Hyla arborea, BB=Bombina bombina, PF=Pelobates fuscus, BU=Bufo bufo, 92=1992, 93=1993).

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			1992				1993						
	S	is	pr	prey/stomach				prey/stomach					
	mean	SD	mean	SD	n	mean	SD	mean	SD	n			
R. esculenta c.	0.18	0.20	12.5	4.56	76	0.15	0.18	8.7	4.56	110			
R. arvalis	0.21	0.23	6.2	4.56	72	0.20	0.20	8.8	4.56	139			
H. arborea	/	/	/	/	/	0.16	0.25	2.7	4.56	58			
B. bombina	/	1	1	/	1	0.30	0.30	47.4	4.56	35			
P. fuscus	0.16	0.24	7.0	4.56	43	0.26	0.27	5.2	4.56	62			
B. bufo	0.53	0.22	73.4	4.56	24	0.60	0.25	58.0	4.56	23			

TABLE 2. Intraspecific similarities, S_{is} (mean, SD) and number of items per stomach (mean, SD, n) in six anuran species in Diás Island, Hungary.

For *Rana arvalis* the minimum sample sizes were 20 and 36 in the two years.

Hyla arborea occupies different microhabits from the ground to the top of vegetation implying that its diet is variable. However its diet was less diverse than that of *Rana* species (Kovács & Török, 1992). The smallest number of items per stomach was found in this species. As with the *Rana* species, a relatively large number of individuals (32) was needed to estimate the 95% limits of diet diversity in *Hyla arborea*.

Bombina bombina, the most aquatic species, fed mainly on collembolans living on the surface of the water and, to a lesser extent, on amphipods and beetles. The average number of prey items per stomach was quite high. Studying the sibling species, *B. variegata*, Kuzmin (1990) found a much smaller prey number per specimen than we did, although the most abundant prey types showed a great similarity to the prsent data. Studies conducted in Ukraine and South Russia reported a rather different diet composition (Medvedev, 1974), Goncharenko *et al.*, 1978; Tertyshnikov & Gorovaya, 1982). Coleoptera, Lepidoptera larvae and



FIG. 2. Relationship between slope (a) and asymptotic diversity $(H_{\rm lim})$ of the hyperbolic curves fitted to data of rarefaction analysis (four and six species in 1992 and 1993, respectively are pooled).

Diptera were the dominant prey species, but collembolans were absent. In Diás Island the diversity was 1.79, which is relatively low, and the 95% limit was reached at 13 specimens. The high number of prey per stomach and the high similarity in the diet between individuals meant that diversity could be estimated from a small sample size in this species.

We studied two nocturnal species, *Pelobates fuscus* and *Bufo bufo*. The diet of the former was much more diverse than that of the latter (Kovács & Török, 1992). The number of prey items per stomach was also comparatively low. The minimum sample size was 24 individuals for *Pelobates fuscus* but only 8 for *Bufo bufo* (Fig. 1.). The latter species foraged mainly for patchily distributed prey (more than 60% of its diet consisted of ants); therefore the number of prey items per stomach was extremely high (Table 2). As in *Bombina bombina*, too few specimens were obtained to compute the diversity for this species. Medvedev (1974) reported a high preference for Coleoptera in *Pelobates fuscus* implying a very low diet diversity. Other studies also showed small and highly aggregated



FIG. 3. Slope (crosses) and asymptotic diversity (triangles) of the hyperbolic curves fitted to data of rarefaction analysis in relation to intraspecific similarity (S_{is}) (four and six species in 1992 and 1993, respectively are pooled)

prey types in *Bufo bufo* (Medvedev, 1974; Wheater, 1986; Kuzmin, 1990).

The intraspecific variance of diet diversity can influence the shape of the individual diversity curve. One of the extremes is when all specimens eat different food types, the other is when all individuals eat the same types. In the first case the curve increases steeply, while in the other case the diversity is independent of the sample size and shows a horizontal line.

Intraspecific diet similarity was also computed among individuals. It ranged between 0.15 and 0.30, except for *Bufo bufo* where similarity reached 0.60 (Table 2). The negative correlation (r=-0.93, t=7.21, P=0.0001, n=10) between similarity and the asymptotic value in the rarefaction curves reflects the fact that diversity can be estimated from small sample sizes in species with a low asymptotic value (Fig. 3.).

The correlation between similarity and slope of rarefaction curve was positive and significant (r=0.80, t=3.70, P=0.0060, n=10).

In conclusion, this study has demonstrated how diet diversity depends on sample size in six anuran species. Rarefaction analysis allows the estimation of a minimum sample size in all species. The higher the diversity, the steeper the slope of the saturation curve. The diet diversity negatively depended on intraspecific diet similarity.

This analysis is applicable in preparing long-term field studies on the diet of any group of animals. The first step is to collect a large sample of each species - in the case of amphibians, approximately 60-80 individuals. After identifying the food items the diversity of the diet is calculated. These values are then used to compute the rarefaction curve and the asymptote of hyperbolic function fitted on the rarefaction curve. Where diversity reaches 95% of the asymptote value the minimum sample size can be determined. Sample sizes around this minimum value can be obtained for subsequent sampling periods.

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REPRODUCTIVE ACTIVITY AND SEXUAL DIMORPHISM OF *LIOLAEMUS* MULTIMACULATUS (SAURIA: TROPIDURIDAE)

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The lizard *Liolaemus multimaculatus* inhabits coastal sand dunes of the Buenos Aires Province of Argentina, and exhibits reproductive activity during the spring and early summer months. Females had vitellogenic follicles and oviductal eggs between September and December, and the size at maturity was 48.2 mm (snout-vent length). Five females with yolked follicles as well as eggs were collected in November, indicating that at least some females can produce more than one clutch per reproductive period. Mean clutch size was 4.2 eggs (range 3 to 7) and clutch size was positively correlated with female body size. In males, testicular diameter peaked in August (late winter) and September (early summer) and declined in January (mid-summer). Testis size increased gradually from January onwards. Individual males were seen more frequently during the reproductive period than females. Hatchlings were first seen in February and March and the smallest measured 26.7 mm (snout-vent length). *Liolaemus multimaculatus* males were larger than females in several morphological traits: snout-vent length, he ad length, distance between fore and hind limbs, tibio-fibulla, hand and foot lengths.

INTRODUCTION

Reproductive patterns, growth rates and survivorship are the principal life history traits of an organism (Stearns, 1992). Recently, the reproductive cycles of temperate lizards in Argentina have begun to be studied (Fitzgerald et al. 1993; Aun & Martori, 1994; Martori & Aun, 1994; Cruz, 1994a, b, 1995), including some of the tropidurid Liolaemus species (Ramirez Pinilla, 1991a, 1992). Reproductive cycles of the wiegmanni group (Etheridge, 1995) which includes L. multimaculatus and other arenicolous forms of Argentina and South America are known, specifically for L. wiegmanni (Ramirez Pinilla, 1991b; Martori & Aun, unpublished data), L. scapularis (Ramirez Pinilla, 1994) and L. lutzae, a tropical lizard from south-eastern Brazil (Rocha, 1992). For all these species, the reproductive pattern was described as a cyclic spring-summer pattern with variations in clutch size or in the number of clutches per season. Recently, Ramirez Pinilla (1994) pointed out that in spite of its poorly known cycle, observations of male and female individuals of L. multimaculatus suggest they should also exhibit reproductive activity in spring and early summer.

The aim of this paper is to describe the reproductive activity, size at maturity and clutch size of *L. mutimaculatus*, and also to examine sexual dimorphism in the population under study. Results will be compared to related species.

MATERIAL AND METHODS

Liolaemus multimaculatus is a small lizard with a brownish dorsum, with small dark spots and blue scales between armpit and groin. It is an endemic species from

the coastal sand dune habitats of Buenos Aires and Río Negro Provinces of Argentina (Gallardo, 1977; Cei, 1993). In a recent taxonomic review (Etheridge, 1993) the forms *L. m. multimaculatus* and *L. m. riojanus* from the sandy flats of La Rioja and San Juan Provinces (Cei, 1979) were regarded as two different species.

STUDY AREA

Fieldwork was carried out at Mar del Sur (38°21'S; 57°59'W) in the south-east of Buenos Aires Province, Argentina. Mean annual temperature is 13.5°C with maximum average in January (21°C) and minimum in July (7.5°C). Annual average rainfall is over 830 mm and there is no marked wet or dry season, although precipitation is at its highest level in March and lowest in August, with 105 mm and 50 mm respectively. The vegetation at the site consisted mainly of Spartina ciliata and Panicum racemosum on the foredunes and short grass communities of Bothriochloa sp., Lagurus ovatus, Adesmia incana and Ambrosia tenuifolia stabilizing backdunes. Another lizards in the study area was L. gracilis, which occupied foredunes along with L. multimaculatus. Stenocercus pectinatus and Ophiodes vertebralis occurred in the area, but were restricted to backdune habitats (Vega, 1994).

The study site was visited once every month, from September 1984 to December 1985. During each visit, a census between 11.00 and 12.00 hrs was made by walking a 15 ha strip along coastal sand dunes, searching for lizards. For each lizard, age (adult or juvenile) was recorded, but sex was difficult to identify. Before each census, air temperature (100 cm above ground) was measured with a Miller-Weber thermometer to the nearest 0.1°C. From January to December 1985, in ad-

dition to the census, an attempt was made to capture individuals by hand. A total of 73 specimens (adults: 33 males, 29 females; juveniles: 6 males, 7 females) was collected. All these specimens were killed with ether minutes after capture, and within a few hours were injected with 10% formalin and stored in 70% ethyl alcohol. Voucher specimens were deposited at the Herpetological Collection of Vertebrate Laboratory, Universidad Nacional de Mar del Plata. Measurements were made using a digital calliper (accuracy 0.01 mm) and included snout-vent length (SVL), head length (from posterior edge of auricular opening to rostral scale), head width (between corners of the mouth), distance between fore and hind limbs, humerus length (from elbow to axilla), radius-ulna length (from elbow to the internal angle between hand and forearm), hand length (including fourth toe-nail), femur length (from knee to groin), tibio-fibula length (from knee to the internal angle with the foot), foot length (including fourth toe-nail) and tail length.

Specimens were dissected and gonads removed. Sex and age class (juvenile or adult) were noted. Females were considered adults if they had yolked follicles (YF) over 2 mm that were opaque and yellow, oviductal eggs (OE) or distended oviducts, in which case left oviduct width (OW) was measured. For males, sexual maturity was based on the presence of enlarged testes and convoluted epididymes, and left testis length (TL) and width (TW) were measured. All these measurements were made using an ocular micrometer of a binocular microscope.

In statistical analyses, distribution and variances of the data sets were examined, and transformations were performed as necessary to ensure that the assumptions of the tests being applied were not violated. The effects of SVL were removed with ANCOVAs when slopes among groups were homogeneous at the P < 0.05 alpha level. Means are given ± 1 SD (Zar, 1984).

RESULTS

FEMALE REPRODUCTIVE CYCLE AND CLUTCH SIZE

The smallest reproductive female with yolked follicles measured 48.2 mm SVL and the biggest measured 63.2 mm SVL, while the smallest female with eggs measured 51.2 mm SVL. Mean SVL of females showing evidence of reproduction was 54.6 mm \pm 3.97 (*n*=29). The smallest female size with distended oviducts (OW = 2.5 mm) but without yolked follicles or oviductal eggs, was 54.8 mm SVL.

Principal events of the adult (individuals > 48.2 mm SVL) female cycle were the following: in September 100% of females (n=1) had yolked follicles, in November, 62.5% (n=8) of females had simultaneously yolked follicles and oviductal eggs while another 37.5% (n=8) had oviductal eggs. In December, 12.5% (n=16) of the females had oviductal eggs and 25% (n=16) yolked follicles. In January and February (n=1 and 3 respectively) there was no evidence of reproductive activity.



FIG. 1. Reproductive events of male *Liolaemus multimaculatus*. Mean values of testis diameters represented by bold lines. 1 SD represented by vertical lines.

Clutch size, based on the number of oviductal eggs in both oviducts, ranged from 3 to 7 (mean = 4.2 ± 1.22 , n = 10). Clutch size was positively correlated with female size (SVL) (Spearman's r = 0.72, n = 10, P < 0.03).

MALE REPRODUCTIVE CYCLE

The smallest male with obviously enlarged testes was 47.5 mm SVL (TL = 4.5 mm, TW = 3 mm) and the largest reproductive male was 69.6 mm SVL (TL = 8 mm, TW = 6 mm). The mean SVL of males showing evidence of reproduction was 59.1±4.42 mm SVL (n= 30). The highest values of width and length of testis were observed in August and September, decreasing in the following months until testis sizes were at their minimum sizes in January. Testis size began to increase again in February and March (Fig. 1). There were significant differences in adjusted TW using least squares means, for the months evaluated (ANCOVA: F = 13.03P < 0.0001, df = 4,21, n = 27; homogeneity of slopes: df = 4,17, F = 0.01, P < 0.05) and Tukey Multiple Comparisons Test found significant differences (P < 0.05) between December and all months except February, and between February and August. Values of September, January and March were not considered in the analysis because only one specimen was in the sample.



FIG. 2. Size-frequency distributions for male and female *Liolaemus multimaculatus*.

REPRODUCTION IN LIOLAEMUS

TABLE 1. Survey and capture data of Liolaemus multimaculatus at Mar del Sur. * air temperature during census

			Number of individuals									
		Cen	sused	Captured								
Date	T°C*	adult	juvenile	adult male	adult female	juvenile						
September 1984	11	0	0	0	0	0						
October 1984	17	0	0	0	0	0						
November 1984	30	12	0	0	0	0						
December 1984	17	0	0	0	0	0						
January 1985	27	2	0	1	1	0						
February 1985	26	9	1	2	3	1						
March 1985	26	1	3	1	0	1						
April 1985	18	0	0	0	0	0						
May 1985	21	1	0	0	0	0						
June 1985	18	0	0	0	0	0						
July 1985	12	0	0	0	0	0						
August 1985	21	7	0	6	0	0						
September 1985	11	3	0	1	1	0						
October 1985	28	6	0	4	0	0						
November 1985	25	16	2	8	8	2						
December 1985	27	38	9	10	16	9						

REPRODUCTIVE ACTIVITY

Individuals of *L. multimaculatus* emerged from over-wintering in August and ceased activity in late March. The numbers of individuals seen active during censuses were positively correlated with air temperatures on the census days (Spearman's r= 0.74, n = 16, P < 0.002).

Lizards were caught in the same proportion as were seen (Table 1). Between April and July, lizard activity was not observed except for one individual seen in May, but no captures were made during these months. Captures in August and October were exclusively comprised of males. The highest number of females were caught in November and December, comprising 82% of total female captures (Table 1).

The two smallest sized individuals, probably hatchlings, were collected in February (male, SVL = 26.7 mm) and March (male, SVL = 29.5), but one individual of approximately the same body size was seen in January in Costa Bonita locality, 60 km south of Mar del Sur, though hatching period would seem to occur during mid-summer (January, February). Larger juveniles or subadult lizards collected ranged from 39.1 mm to 46.4 mm SVL (n = 11).

SEXUAL DIMORPHISM

L. multimaculatus exhibited sexual size dimorphism, with males attaining larger sizes than females (Mann-Whitney *U* Test: $z_{30,29} = 3.74$ P = 0.0001) (Fig. 2). Analyses of covariance used to remove the effects of SVL revealed that males had significantly larger heads than females (see Table 2 for statistics). Similarly, the distance between forelimbs and hindlimbs,

length of tibio-fibula, and lengths of hands and feet were significantly larger in males than in females. There were not found to be significant differences between sexes in morphological traits such as head width and lengths of humerus, radius-ulna, femur and tail (Table 2).

Sexual dichromatism was only evident in ventral surface. Females possessed an immaculate venter, whereas males showed brown spots on the throat and ventral surface. Ventral spots were more diffuse in juvenile males. Males had also yellowish precloacal pores that were lacking in females.

DISCUSSION

The reproductive cycle of L. multimaculatus is seasonal in spring (September) and early summer (December). Testicular diameter was maximum in late winter (August) and early spring (September) and minimum in mid-summer; in late summer it began to increase gradually. Although the middle and late summer sample was small, it seems that testicular regression would be at a maximum at mid-summer and recrudescence would occur in early autumn. This testis cycle was also observed in other species of the wiegmanni group (Ramirez Pinilla, 1991b, 1994; Rocha, 1992; Martori & Aun, unpublished data). Maximum testicular diameters were synchronized with the presence of vitellogenic follicles in females through September to November, but not in December. First copulations probably occur in August and September.

The simultaneous occurrence of oviductal eggs and enlarged vitellogenic follicles in females captured in November suggests that some females produce more than one clutch per season. Multiple clutches are

			ANCOVA	A results
	Mean (SD)	Ν	<i>F</i> -ratio	Р
Head length				
males	14.16 (1.00)	30	5.22	0.026
females	12.93 (0.98)	29		
Head width				
males	10.51 (1.02)	30	3.64	0.061
females	9.54 (0.65)	29		
Distance between lir	mbs			
males	26.00 (2.93)	28	25.48	0.000
females	25.87 (3.09)	25		
Humerus length				
male	5.64 (0.85)	28	0.13	0.719
females	5.12 (0.43)	25		
Radius-ulna length				
males	7.59 (0.80)	28	0.69	0.409
females	6.89 (0.61)	25		
Hand length				
males	10.27 (0.66)	30	50.74	0.000
females	8.80 (0.56)	29		
Femur length				
males	8.44 (0.88)	28	0.17	0.677
females	7.91 (0.75)	25		
Tibio-fibula length				
males	9.39 (1.01)	28	4.14	0.047
females	8.12 (0.92)	25		
Foot length				
males	16.88 (0.96)	30	21.78	0.000
females	15.14 (1.07)	29		
Tail length				
males	55.86 (7.69)	25	0.25	0.615
females	51.68 (5.59)	26		

TABLE 2. Results of tests for differences in the mean size of several morphological traits of male and female *Liolaemus multimaculatus*. The effects of SVL were removed using ANCOVA. All measurements are in mm.

known to occur in L. wiegmanni from central and chacoan regions of Argentina (Ramirez Pinilla, 1991b; Martori & Aun, unpublished data), in L. scapularis from north-western Argentina (Ramirez Pinilla, 1994) and in L. lutzae from south-eastern Brazil (Rocha, 1992). Mean clutch size of L. multimaculatus (4.2 eggs) was similar to that observed for L. wiegmanni from the chaco (4.5 eggs) (Ramirez Pinilla, 1991b) and L. scapularis (4.3 eggs) (Ramirez Pinilla, 1994), but about one egg less on average than in L. wiegmanni from central regions of the country (5.4 eggs) (Martori & Aun, unpublished data). Mean clutch size of L. multimaculatus at our site was greater than that observed for L. lutzae (2.0 eggs), but L. lutzae produces two or three clutches over a longer reproductive period (Rocha, 1992). For all these species, female size at maturity ranged from 40.2 mm to 51.5 mm SVL (Ramirez Pinilla, 1991*b*, 1994; Rocha, 1992; Martori & Aun, unpublished data), and it is probably associated at the species body size. Clutch size was positively correlated with female body size, a pattern commonly observed in other tropidurid species of Argentina (Ramirez Pinilla, 1994; Cruz, 1995; Martori & Aun, unpublished data). The above mentioned and additional reproductive data of other *Liolaemus* species (Cei, 1993) indicate that a seasonal cycle with an average clutch size of 4 to 6 eggs laid in more than one clutch per reproductive period, would seem to be a life history trait that emerges in oviparous *Liolaemus* species of subtropical and temperate regions of Argentina.

The activity period of *L. multimaculatus* extends through late winter (August), spring (September to December) and late summer (March), while autumn (April) and early and mid-winter (June, July) is the in-

active period. This seasonal and thermic pattern of activity is common in temperate reptiles (Huey, 1982). As the highest rates of observations and captures of females were in late spring, coinciding with their ovigerous condition, it is presumed that females were more active at this time, possibly searching for nesting sites. In contrast, the numbers of males captured monthly in spring and early summer were more constant. This kind of differential activity between sexes, especially the high activity rates of males during the reproductive season, was observed in many lizard species (Stamps, 1983).

Males of *L. multimaculatus* were larger than females in several body traits. This sexual dimorphism may be related to sexual selection, as occurs in most territorial iguanian lizards (Stamps, 1983), or to natural selection by means of intraspecific niche divergence (Schoener, 1977, 1986), but more behavioural and ecological data are needed to differentiate between these hypotheses. Ventral sexual dichromatism of *L. multimaculatus* contrasts with dorsal sexual dichromatism seen in most *Liolaemus* species, that may vary from a different to a paler or less strong pattern for the females (Cei, 1993). In *L. multimaculatus* both males and females are highly dorsally cryptic and match the texture and colour of sandy substrate.

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ON PHYLOGENETIC RELATIONSHIPS WITHIN *DENDROTRITON* (AMPHIBIA: CAUDATA: PLETHODONTIDAE): IS THERE SUFFICIENT EVIDENCE?

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Previous phylogenetic analyses of the relationships among five Central American salamanders of the genus Dendrotriton are reviewed. The available data was reanalysed using parsimony under a variety of analytical treatments. The results are highly sensitive to (1) the coding method used to convert quantitative characters into discrete character states; (2) different scalings (weighting) of multistate characters; and (3) the omission or inclusion of potentially problematic characters. Explorations of length differences between most parsimonious trees and selected less parsimonious alternatives reveal that under each treatment, most parsimonious trees are only marginally more parsimonious than alternatives and that Bremer support for the clades occurring in MPTs is always low. Tree length distributions are not highly left-skewed as would be expected of phylogenetically informative data. These analyses suggest that there is little phylogenetic signal in the available data and that these data provide little basis for well supported phylogenetic inferences. Both parsimony and compatibility-based randomization tests confirm this interpretation. The null hypotheses that the data are not significantly different from phylogenetically uninformative randomly permuted data cannot be rejected for any of the analytical treatments. Given failure to reject the null hypothesis, phylogenetic hypotheses for Dendrotriton based on the available data are uncompelling. Additional data are needed. Results of the randomization tests are consistent with the view that there has been extensive homoplasy in bolitoglossine salamanders.

INTRODUCTION

Dendrotriton was established by Wake & Elias (1983) for five nominate species of Central American salamanders that were previously considered part of the bromeliacia species group of Chiropterotriton beta. Phylogenetic relationships among these species were first investigated by Lynch & Wake (1975) who used numerical techniques to produce three different phylogenetic hypotheses. Relationships within Dendrotriton were also briefly addressed, as part of a broader study of the Chiropterotriton beta group, by Lynch & Wake (1978). More recently, Collins-Rainboth & Buth (1990) identified a number of problems with Lynch & Wake's (1975) original study, the most important of which they considered (p. 956) "relate to the limitations of the programs available" at that time. They sought an improved estimate of the phylogeny of *Dendrotriton* through the application of more recent parsimony analysis software to a revised interpretation of Lynch & Wake's (1975) data, and they presented a unique most parsimonious tree (MPT), as their single best estimate of the phylogeny of Dendrotriton. Here I review these studies, and show through renalyses of both Lynch & Wake's (1975) and Collins-Rainboth & Buth's (1990) interpretations of the data, and by the application of randomization tests, that no hypothesis of phylogenetic relationships within Dendrotriton is well supported by the available data.

LITERATURE REVIEW

Lynch & Wake (1975) compiled data on variation in 11 features (Table 1), six morphometric (1-5, 8), two

meristic (6-7), and three qualitative osteological (9-11), that they used for inferring phylogenetic relationships within Dendrotriton. To convert quantitative data into discrete characters, they scored taxa with distinct character states if their sample means (standardized on body length if correlated with it) were statistically significantly different at a level of P < 0.1. Converted into discrete characters, their interpretation of the data yielded four binary and seven ordered multistate characters (Table 2). They made a priori assessments of character polarity, using the outgroup criterion, for five of their characters (7-11). In addition, they treated the median character state of the remaining morphometric and meristic characters as primitive, based on the assumption that the ancestral species was 'generalized'. Lynch & Wake (1975) experimented with a procedure suggested by Colless (1967) and termed condensation by Crovello (1968), scaling by Swofford (1985) and by Farris (1990) and ranging by Collins-Rainboth & Buth (1990), intended to give each character equal total weight in numerical analyses. They employed two methods of scaling characters, here termed simple and bidirectional approaches respectively. In the simple approach, the weight of each (adjacent) character state transition is the inverse of the one less than the number of character states of that character, so that all characters have a total weight of unity. In the bidirectional approach, deviations from the assumed primitive condition in different directions each have a total weight of unity.

For their phylogenetic analyses, Lynch & Wake (1975) constructed trees rooted on a hypothetical an-

TABLE 1. Variation in seven morphometric, two meristic and three osteological characters across the five species of *Dendrotriton*. The morphometric data provided are the means and (in parentheses) the 95% confidence intervals of the means. After Lynch & Wake (1975) and incorporating the corrections of Collins-Rainboth and Buth (1990). A = absent, P = present, p = minutely or occasionally present. ¹Reported as present in 2 of 15 specimens (Lynch & Wake, 1975). ²Reported as present in an additional specimen by Lynch & Wake (1978).

							Av. confidence
	Character	bromeliacia	cuchumatanus	megarhinus	rabbi	xolocalcae	interval
	sample size	42	9	18	17	38	-
1	Standard length	30.2	28.8	29.7	32.3	30.1	-
2	Tail length	33.2 (0.70)	29.7 (1.20)	32.4 (0.75)	33.2 (1.95)	31.1 (0.65)	1.05
3	Head width	4.60 (0.075)	4.72 (0.115)	4.86 (0.060)	4.73 (0.080)	4.83 (0.065)	0.079
4	Combined limb length	14.9 (0.20)	15.5 (0.25)	14.7 (0.25)	15.7 (0.30)	15.0 (0.20)	0.24
5	Foot width	2.76 (0.065)	2.80 (0.255)	3.07 (0.060)	2.91 (0.110)	2.96 (0.075)	0.113
6	Maxillary teeth	31.7 (1.30)	40.1 (5.10)	49.8 (1.65)	41.9 (2.70)	44.6 (2.40)	2.63
7	Vomerine teeth	10.1 (0.70)	12.7 (2.15)	10.9 (1.05)	17.1 (2.90)	19.9 (0.950)	1.15
8	Nostril diameter	1.89 (0.050)	1.47 (0.100)	2.27 (0.065)	0.72 (0.125)	0.21 (0.020)	0.072
	sample size (osteology)	15	1	2	6	2	
9	Septomaxillae	\mathbf{A}^{1}	A ²	Р	р	А	-
10	Preorbital process of vomer	А	А	А	Р	Р	-
11	Columellar process	р	Р	Р	А	р	-

cestor using Wagner parsimony (Kluge & Farris, 1969), with either simple or bidirectional scaling, and the now little-used WISS (weighted invariant step strategy) method of Farris et al. (1970), which prohibits evolutionary reversals, using only bidirectional scaling. They also suggested that three of their characters might not be evolutionarily independent, noting that high vomerine tooth counts (character 7) are associated with small nostril sizes (character 8) and the presence of a (dentigerous) preorbital vomerine process (character 10). In view of these concerns, they performed parallel analyses either including or omitting characters (7 and 10). Their six analyses, yielded three distinct trees (Fig. 1, Trees A, B, and C), with the inferred results depending upon method of analysis, the scaling approach used, and the inclusion or exclusion of potentially non-independent characters. The strict component consensus of these three trees is completely unresolved, but there is a single strict reduced cladistic consensus (Wilkinson, 1994) that expresses the agreement among the three trees that D. megarhinus is always more closely related to D. bromeliacia than to D. xolocalcae (Fig. 2).

Lynch & Wake (1975) did not advocate any single, best or preferred phylogenetic hypothesis in the face of their varied results. They attributed the instability of their inferences in part to D. megarhinus, noting that it (p. 39) "is, on the average, the most distinctive in overall morphology, i.e. it has the greatest mean phenetic separation from the other species", and that consequently its position in their trees "tends to shift with practically every modification of the clustering procedure". This is not borne out by the strict reduced cladistic consensus (Fig. 2) which indicates that it is D. cuchumatanus and D. rabbi that have variable positions relative to a more stable phylogenetic framework provided by the other three taxa. They also correctly concluded that (p. 38) "a number of morphological convergences or reversals or both have occurred during the evolution of the group." Most importantly, Lynch & Wake (1975:41) emphasized the limitations of their study by suggesting that "Further refinement of our knowledge of the relationships within this group will depend upon the examination of new suites of characters".

TABLE 2. Lynch & Wake's (1975) data matrix for the five species of *Dendrotriton* and a hypothetical ancestor. The presentation is slightly modified from, but analytically equivalent to Lynch & Wake's (1975: Table 1) bidirectional scaling.

					C	haracte	ers				
Taxa	1	2	3	4	5	6	7	8	9	10	11
Hypothetical Ancestor	4	0	4	0	4	4	0	0	0	0	0
bromeliacia	4	4	0	0	0	0	4	3	4	4	4
cuchumatanus	0	0	4	4	0	4	4	2	4	4	0
megarhinus	4	4	8	0	8	8	4	4	0	4	0
rabbi	8	4	4	4	4	4	0	1	2	0	4
xolocalcae	2	0	4	0	2	2	0	0	4	0	2



FIG. 1. Four phylogenetic hypotheses for *Dendrotriton* (Trees A - D) proposed in previous studies and the analytical conditions under which they were recovered. Numbers in parentheses indicate omitted characters: A, (Lynch & Wake, 1975) Wagner parsimony, simple bidirectional; B, (Lynch & Wake, 1975) Wagner parsimony, simple (7,10) WISS, bidirectional (7,10); C, (Lynch & Wake, 1975) WISS, bidirectional; D, (Collins-Rainboth and Buth, 1990) Wagner parsimony, no scaling.

Lynch & Wake (1978) presented a tree depicting relationships among eight species of *Chiropterotriton* Beta including the five species of *Dendrotriton* and three species that were subsequently transferred to *Nototriton* (Wake & Elias, 1983), and which, according to Wake & Elias (1983), are not closely related to *Dendrotriton* (but see Sessions & Kezer, 1991). Lynch & Wake's (1978) focus was primarily upon these latter three species, and their tree was not based on any numerical analysis. The relationships within *Dendrotriton* correspond to Tree C of Fig. 1, but no reason for this choice was presented. Lynch & Wake's (1978) study is of interest here primarily for their report that a second



FIG 2. Unique primary reduced cladisitic consensus tree for the Trees A-C of Fig. 1.

specimen of *D. cuchumatanus* has a pair of distinct septomaxillaries, which has implications for the coding of character 9 of Lynch & Wake (1975).

Collins-Rainboth & Buth's (1990) interpretation and analysis of the data differs from those of Lynch & Wake (1975) in several important respects. Firstly, they did not include standard length (character 1). Secondly, for the remaining morphometric and meristic characters, they used a gap-coding method (Archie, 1985), in which a gap was the average of half the 95% confidence interval of the standardized means of the character for each species. As a result of this coding procedure, they were unable to detect any distinct character states in character 5 (foot width), but detected extra character states in characters 2, 6 and 7. Thirdly, they accepted Lynch & Wake's (1975) assessments of character polarity based on the outgroup criterion, but coded the hypothetical ancestor in their data matrix with missing entries for the remaining characters. The latter characters were therefore unpolarized in their analyses and have no effect upon the placement of the root in resulting trees. Fourthly, they did not employ any scaling of characters, so that each (adjacent) character state transition has equal weight independent of the numbers of character states within characters. Fifthly, they used only Wagner parsimony.



FIG 3. Summary of the parsimony analyses of the five treatments of Collins-Rainboth & Buth's 1990 data including the extra length of trees A-D. Where there are multiple MPTs for a treatment the tree shown is the strict component consensus. b, *D. bromeliacia*; c, *D. cuchumanatus*; m, *D. megarhinus*; r, *D. rabbi*; x, *D. xolocalcae*; CI, consistency index; RI, retention index. Numbers next to branches are Bremer support values.

Collins-Rainboth & Buth (1990) also corrected three typological errors in Lynch & Wake's (1975) appendix. Of these changes, only one, relating to the condition of the columellar process of the opercular apparatus (character 11), leads to any modification of Lynch & Wake's (1975) data matrix. Lynch & Wake (1975) reported that 3 of 13 specimens of *D. bromeliacia* possessed a columellar process similar to the small but discrete processes of *D. xolocalcae*, but coded the process as absent. Collins-Rainboth & Buth (1990) changed the coding of *D. bromeliacia* from absent to the intermediate state of occasionally or minutely present.

Despite parallel intraspecific variation, Collins-Rainboth & Buth (1990) proposed no modification to character 9, the condition of the septomaxillae. Their coding, like that of Lynch & Wake (1975), includes the three states: absent, minutely or occasionally present, and present. Lynch & Wake (1975) report that this bone is present in only two of six specimens of *D. rabbi* ex-

amined and it is coded as minutely or occasionally present in both Collins-Rainboth & Buth (1990) and Lynch & Wake (1975). In contrast, D. bromeliacia, for which Lynch & Wake (1975) reported that two of fifteen specimens had septomaxillae (large in one specimen and small in the other), was coded as absent in both studies. Thus there is some inconsistency, both in Lynch & Wake (1975) and Collins-Rainboth & Buth (1990) with regard to how intraspecific variation in the columellar process of the opercular apparatus and septomaxillae were handled. Collins-Rainboth & Buth's (1990) coding of the condition of character 9 also takes no account of Lynch & Wake's (1978) observation of septomaxillae in D. cuchumatanus, or their conclusion that (p. 294) "Septomaxillaries have not been observed in C. xolocalcae, but experience has shown that these bones are found in at least a small percentage of other members of the group, and the absence here may reflect small sample size." Given that the conditions of both the collumelar process of the opercular apparatus and the septomaxillae are known to vary within species, and that sample sizes are small for most of the species, it might be concluded that the boundaries between the character states of these characters and the somewhat arbitrary partitioning of variable species into these character states, are unlikely to reflect phylogeny.

Collins-Rainboth & Buth (1990) used PAUP version 2.4. (Swofford, 1985) to analyse their data matrix. Multistate characters were treated as linear ordered transformation series (i.e. they used Wagner parsimony) and two analyses utilizing either the full data matrix or omitting the osteological characters (9-11) were performed. The latter analysis was performed because, according to Collins-Rainboth & Buth (1990), the original study had explored these treatments (p. 958) "because of the limited number of individuals examined for the latter characters." This is inaccurate. As described above, Lynch & Wake (1975) performed parallel analyses with all the characters or without two of the characters (7 and 10). One of these excluded characters is osteological (10) the other is meristic (7), and the analysis without these characters was performed because Lynch & Wake (1975) considered that they might form part of a functional complex with another character (8). Of course, concern over small sample sizes, as expressed by Lynch & Wake (1975, 1978), may also provide good grounds for experimental omission of the osteological characters.

Both parsimony analyses performed by Collins-Rainboth & Buth (1990) yielded the same single MPT (Fig. 1: Tree D), different from the trees reported by Lynch and Wake (1975), though consistent with the reduced cladistic consensus of the latter trees. They reported that tree lengths among the 105 possible tree topologies (using all the characters) ranged from 29 to 38, that the frequency distribution of lengths of all topologies was not significantly skewed ($g_1 = 0.080$) or

Characters									
2	3	4	5	6	7	8	9	10	11
?	?	?	?	?	3	0	2	1	2
2	0	0	0	0	0	3	0	0	1
0	1	1	0	1	1	2	0	0	2
2	2	0	0	3	0	4	2	0	2
2	1	1	0	1	2	1	1	1	0
1	2	0	0	2	3	0	0	1	1
	2 ? 2 0 2 2 1	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c cccccc} \hline \\ \hline \hline 2 & 3 & 4 & 5 & 6 & 7 \\ \hline \hline 2 & 7 & 7 & 7 & 7 & 3 \\ \hline 2 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 1 & 0 & 1 & 1 \\ 2 & 2 & 0 & 0 & 3 & 0 \\ 2 & 1 & 1 & 0 & 1 & 2 \\ 1 & 2 & 0 & 0 & 2 & 3 \end{array}$	$\begin{array}{c ccccc} \hline \\ \hline \hline 2 & 3 & 4 & 5 & 6 & 7 & 8 \\ \hline 2 & 0 & 0 & 0 & 0 & 0 & 3 \\ \hline 2 & 0 & 0 & 0 & 0 & 0 & 3 \\ 0 & 1 & 1 & 0 & 1 & 1 & 2 \\ 2 & 2 & 0 & 0 & 3 & 0 & 4 \\ 2 & 1 & 1 & 0 & 1 & 2 & 1 \\ 1 & 2 & 0 & 0 & 2 & 3 & 0 \end{array}$	$\begin{array}{c ccccc} Characters \\ \hline \hline 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 \\ \hline ? & ? & ? & ? & ? & 3 & 0 & 2 \\ 2 & 0 & 0 & 0 & 0 & 0 & 3 & 0 \\ 0 & 1 & 1 & 0 & 1 & 1 & 2 & 0 \\ 2 & 2 & 0 & 0 & 3 & 0 & 4 & 2 \\ 2 & 1 & 1 & 0 & 1 & 2 & 1 & 1 \\ 1 & 2 & 0 & 0 & 2 & 3 & 0 & 0 \end{array}$	$\begin{array}{c ccccc} Characters \\ \hline \hline 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 \\ \hline ? & ? & ? & ? & ? & 3 & 0 & 2 & 1 \\ 2 & 0 & 0 & 0 & 0 & 0 & 3 & 0 & 0 \\ 0 & 1 & 1 & 0 & 1 & 1 & 2 & 0 & 0 \\ 2 & 2 & 0 & 0 & 3 & 0 & 4 & 2 & 0 \\ 2 & 1 & 1 & 0 & 1 & 2 & 1 & 1 & 1 \\ 1 & 2 & 0 & 0 & 2 & 3 & 0 & 0 & 1 \end{array}$

TABLE 3. Collins-Rainboth & Buth's (1990) data matrix for the five species of *Dendrotriton* and a hypothetical ancestor. Character five is invariant and was not included in their analyses or the reanalyses.

different from normal, and that the most parsimonious tree was significantly shorter than the mean tree length. In addition, the three tree topologies reported by Lynch and Wake (1975) (Fig. 1: Trees A-C) were shown to have lower consistency indices using their revised character coding. Given these results, Collins-Rainboth & Buth (1990) presented their MPT as the single best, and preferred, hypothesis of the phylogeny of *Dendrotriton*, and they attributed their resolution of this phylogenetic problem to their retroactive application of newer analytical techniques, without any accumulation of the new data that Lynch & Wake (1975) thought necessary.

It should be apparent from this review, that phylogenetic inferences for *Dendrotriton*, have been based on fairly limited data, and that these inferences appear to be sensitive to variation in (1) how qualitative characters are partitioned into character states, (2) scaling (weighting) of discrete characters, (3) method of analysis, and (4) inclusion or exclusion of characters that may not be independent. The instability of the results suggests that the available data may not provide a sufficient basis for robust inferences of phylogenetic relationships within *Dendrotriton*.

Following a suggestion from Fitch (1979), several workers have explored the frequency distributions of tree lengths supported by real, random and simulated data (e.g. Le Quesne, 1989; Hillis, 1991; Huelsenbeck, 1991) and argued that the skewness of such distributions can be used to assess whether the data contains any useful phylogenetic signal. Both theoretical and empirical studies indicate that data containing strong phylogenetic signal are expected to support a strongly left-skewed tree length distribution. The g₁ statistic of Sokal & Rohlf (1981) provides a measure of skewness, and Hillis (1991) has described critical values for a significantly more left-skewed distribution than that yielded by random, phylogenetically uninformative data. For six taxa, a g, of less than -0.51 indicates that the data is significantly more left-skewed than random data. Collins-Rainboth & Buth's (1990) reported g, for their analysis using all the characters is 0.08, indicating a slight right-skew and an insignificant departure from randomness. Källersjö et al. (1992) are highly critical

of using the skewness of tree length distributions to assess data, and have shown that it can give misleading results. However, g_1 values can be taken as suggestive of properties of the data, and are used in this way here. As we shall see, g_1 suggests conclusions that are fully supported by more direct randomization tests of the quality of the data.

MATERIALS AND METHODS

The potential for the available data to support robust inferences of phylogenetic relationships within *Dendrotriton* was assessed through multiple parsimony analyses using PAUP 3.1.1 (Swofford, 1993), and through the application of parsimony and compatibility-based randomisation tests.

Collins-Rainboth & Buth's (1990) data matrix was analysed, as in their study, without any scaling of characters. Also, as in their study, separate analyses were performed using all the characters and with the osteological characters (9-11) omitted. These two treatments replicate their original analysis. In addition, analyses were performed with the characters (7 and 10) considered potentially interdependent by Wake & Lynch (1975) omitted, with the problematic condition of the septomaxillae (character 9) omitted, or with all three of these characters omitted. Lynch & Wake's (1975) data matrix was reanalysed with the same five combinations of characters included or omitted. Parallel analyses were performed with simple and bidirectional scaling of characters, as in the original, and also without any scaling. The total of 20 analytical treatments used are summarized in Table 4.

All analyses used Wagner parsimony with all multistate characters linear ordered. Topological constraints were used to determine the extra length required to overturn clades common to the MPTs, i.e. the clades decay index (Donoghue *et al.*, 1992) or Bremer support (Bremer, 1988; Källersjö *et al.* 1992), and the comparative lengths of Trees A-D. PAUP also provided consistency and retention indices and g_1 statistics.

Each of the 20 different analytical treatments was used in corresponding parsimony and compatibility-

TABLE 4. Results of parsimony and compatibility-based randomization tests for 20 treatments of Lynch and Wake's (1975) and Collins-Rainboth and Buth's (1990) data.

		Characters		Parsimony		РС	
	Data	Omitted	Scaling	РТР	HER	РТР	IER
1	Lynch & Wake (1975)	None	None	0.62	0.023	0.511	0.000
2	Lynch & Wake (1975)	7 and 10	None	0.30	0.190	0.198	0.000
3	Lynch & Wake (1975)	9 - 11	None	1.00	-0.262	0.889	-0130
4	Lynch & Wake (1975)	9	None	0.46	0.072	0.337	0.050
5	Lynch & Wake (1975)	7, 9 and 10	None	0.91	-0.099	0.955	-0.271
6	Lynch & Wake (1975)	None	Simple	0.40	0.041	0.715	0.055
7	Lynch &Wake (1975)	7 and 10	Simple	0.64	-0.039	0.527	-0.026
8	Lynch & Wake (1975)	9-11	Simple	0.79	-0.111	0.759	-0.124
9	Lynch & Wake (1975)	9	Simple	0.40	0.042	0.464	0.000
10	Lynch & Wake (1975)	7, 9 and 10	Simple	0.91	-0.148	0.768	-0.689
11	Lynch & Wake (1975)	None	Bidirectional	0.31	0.070	0.629	-0.078
12	Lynch & Wake (1975)	7 and 10	Bidirectional	0.49	0.013	0.476	0.006
13	Lynch & Wake (1975)	9-11	Bidirectional	1.00	-0.357	0.753	-0.010
14	Lynch & Wake (1975)	9	Bidirectional	0.35	0.091	0.424	0.008
15	Lynch & Wake (1975)	7, 9 and 10	Bidirectional	0.95	-0.184	0.702	-0.092
16	Collins-Rainboth & Buth (1990)	None	None	0.53	0.025	0.670	-0.041
17	Collins-Rainboth & Buth (1990)	7 and 10	None	0.15	0.025	0.178	0.190
18	Collins-Rainboth & Buth (1990)	9-11	None	0.99	-0.250	0.988	-0.260
19	Collins-Rainboth & Buth (1990)	9	None	0.20	0.220	0.273	0.084
20	Collins-Rainboth & Buth (1990)	7, 9 and 10	None	0.98	-0.220	0.963	-0.245

based randomization tests. Randomization tests allow the comparison of properties of real data (typically a measure of congruence) to be compared to those of similar but phylogenetically uninformative data. By randomly permuting the assignment of character states to taxa, congruence among the characters is reduced to that expected by chance alone but other features of the original data (numbers of taxa, characters, character states, and taxa in each character state) are unaltered. This allows the null hypothesis that the real data are no more congruent than random and phylogenetically uninformative data to be tested. If the null hypothesis cannot be rejected then the data would seem to provide no compelling basis for preferring any phylogenetic hypothesis.

The parsimony-based randomization test used is that developed independently by Archie (1989*a*) and by Faith & Cranston (1991) in which the length of most parsimonious trees (MPTs) supported by the original data is compared to the lengths of MPTs. Tree length is thus used as a measure of congruence. Faith & Cranston's (1991) parsimony permutation tail probability (PTP), defined as the proportion of data sets (original and randomly permuted) supporting MPTs as short or shorter than the original provides a test statistic for the null hypothesis and was determined using the Hennig86 (Farris, 1988) parsimony program and 99 randomly permuted data sets. Archie's (1989*b*) descriptive statistic, the homoplasy excess ratio (HER), was also determined. HER = ($E_L - O_L$)/($E_L - M_L$), where M_L is minimum length if all characters are congruent, E_L is the expected or mean length of MPTs supported by randomly permuted data, and O_L is the observed length of MPTs supported by the real data.

HER is positive if the real data supports MPTs that are shorter than the expected length for randomly permuted data, has a maximum value of unity when the data includes no incongruence, and approaches zero as the level of incongruence approaches that expected for randomly permuted data. Thus values close to zero or negative values indicate that the data show little or no more congruence than expected by chance alone.

Faith & Cranston (1991) suggested that where the focus of the test is ingroup relationships, hypothetical ancestors or outgroups should be excluded from the random permutation, and thus maintained unaltered in all the randomly permuted data sets. I have followed this suggestion, but note that random permutation of the ingroup only will make any single hypothetical ancestor or outgroup essentially random with respect to the remaining taxa and has little or no impact upon test results (pers. obs). Faith & Cranston's (1991) suggestion is expected to be more important in cases where it would preserve non-random relations among multiple outgroups.

The compatibility-based randomization test used was developed independently by Wilkinson (1992) and Alroy (1994), and uses the number of pairwise (in)compatibilities among characters in the data as a measure of congruence. It yields a pairwise compat-



FIG. 4. Summary of the parsimony analyses of the five treatments of Lynch & Wake's (1975) data using simple scaling. Format and abbreviations as in Figure 3.

ibility (PC) PTP test statistic, defined as the proportion of data sets (original and randomly permuted) with as few or fewer pairwise incompatibilities as the original data. The PCPTP is analogous to Faith & Cranston's (1991) parsimony PTP, but differs most importantly in that the measure of congruence is not also a criterion of hypothesis choice. Thus the PCPTP test is tree or hyindependent 1994). pothesis (Alroy, An incompatibility excess ratio (IER) analogous to Archie's (1989b) HER was also determined. IER = $(E_r - E_r)$ O_1/E_1 , where E_1 is the expected or mean number of pairwise incompatibilities of randomly permuted data and O₁ is the number of pairwise incompatibilities for the original data. IER is positive if the real data supports MPTs that are shorter than the expected length for randomly permuted data, has a maximum value of unity when the data includes no incongruence, and approaches zero as the level of incongruence approaches that expected for randomly permuted data. Thus values close to zero or negative values indicate that the data show little or no more congruence than expected by chance alone.

The use of g_1 to assess the phylogenetic signal in cladistic data is somewhat parallel to the parsimony and compatibility-based randomization tests used here. It

differs in that skewness is a much more indirect and unsatisfactory measure of congruence, and that Hillis's (1991) critical values were determined using randomly generated data, whereas random permutation produces critical values that are specific to the data at hand.

To facilitate the compatibility randomization tests and the parsimony analyses with scaling of multistate characters, the data were recoded into an analytically equivalent additive binary form. Interdependent binary factors (either of an originally multistate character or representing character weighting) cannot be incompatible or incongruent with each other and were 'tied' in the randomization tests so that they were randomly permuted with respect to other characters, but not with respect to each other.

RESULTS

Results of the parsimony analyses using Collins-Rainboth & Buth's (1990) interpretation of the data are summarized in Fig. I. The treatments paralleling their original analyses (Treatments 16 and 17) replicate the original results (except for some discrepancies in reported consistency indices and g₁ statistics). In each of these treatments, Tree D is the single MPT and each of Lynch & Wake's (1975) three trees are less parsimonious. Note, however, that using all characters (Treatment 16) Tree A requires only a single extra step, and that Bremer support is low for all clades in the MPT. Excluding the osteological characters (Treatment 17) slightly increases the additional length required by Tree A, but Bremer support remains low for all clades. The same single MPT and parallel statistics are also produced by the omission of just character 9 (Treatment 20).

Treatment 18, in which the potentially interdependent characters identified by Lynch & Wake (1975) are omitted, yields rather different results. There are two MPTs neither of which corresponds to Trees A - D. These trees are unusual in placing *D. megarhinus* as the sister taxon of all other *Dendrotriton*. However, Bremer support is low for the clades common to both MPTs, and none of Trees A - D require more that three additional steps. Similarly, Treatment 19 yields two more distinct MPTs and comparable statistics.

Comparative results for the parsimony analyses using Lynch & Wake's (1975) interpretation of the data, using simple scaling, are summarized in Fig. 4. As in their study, analysis using all characters (treatment 6), yielded Tree A. Paralleling Collins-Rainboth & Buth's (1990) results, omission of the osteological characters (treatment 7) also yields the same unique MPT. Tree A is also one of three MPTs when just character 9 is omitted (treatment 10). However, in all treatments, Bremer support for all clades is low and one or more of Trees B-D are only marginally less parsimonious. In Lynch & Wake's (1975) study treatment 8 (omission of characters 7 and 10) yielded Tree B. In the reanalysis, Tree B is one of three MPTs, and the MPT recovered in treatment 9 represents an additional distinct MPT. Again, neither of these treatments yield MPTs that are much shorter than one or more of their less parsimonious competitors among Trees A-D, and Bremer support for all clades is low. In all treatments g_1 is low.

Essentially similar results were found in the analyses using Lynch & Wake's (1975) interpretation of the data under bidirectional scaling or without scaling (data not shown), with MPTs depending upon which characters are included, always being only marginally more parsimonious than competitors from among Trees A-D, and with all clades having low Bremer support. In all treatments of both Lynch & Wake's (1975) and Collins-Rainboth & Buth's (1990) interpretations of the data, g_1 is either positive or slightly negative reflecting tree length distributions that are never strongly left-skewed as would be expected of phylogenetically informative data.

Results of the parsimony and compatibility-based randomization tests are summarized for all treatments in Table 4. In all cases, both parsimony PTPs and PCPTPs are not significant (> 0.05) and therefore do not allow the null hypothesis that the data are random with respect to phylogeny to be rejected under any of the diverse character coding and scaling (weighting) schemes used. For all analyses, the descriptive HER and IER statistics are close to zero or negative and also betray a lack of congruence within the data that might distinguish them from the expectations for phylogenetically uninformative, randomly permuted data.

DISCUSSION

Collins-Rainboth & Buth (1990:955) considered that Lynch & Wake's (1975) "failure to resolve a single most parsimonious cladogram for this group may not be due to limitations of their data, but rather to confusion with regard to the nature of the analyses and limitations of the algorithms available to them at that time." In particular, they noted that the programs available to Lynch & Wake (1975) required the input of ancestral character states, so that all characters had to be polarized, that they were limited to generating a single MPT rather than finding all such trees, and that the order of data input may have influenced clustering. They believed that their reanalyses solved these problems through the recoding of the data and use of more modern software that was free from these limitations. They aimed to support one of Lynch & Wake's (1975) three trees (Trees A-C), but their analysis yielded a different MPT (Tree D).

A major difference between Collins-Rainboth & Buth's (1990) and Lynch & Wake's (1975) analyses is in the coding of the hypothetical ancestor which was used to root the trees. Collins-Rainboth & Buth (1990) accepted Lynch & Wake's (1975) polarity assessments based on outgroup comparisons but rejected those based on the assumption that the ancestor cx was generalized, although (p. 957) they considered this to be "a reasonable supposition". Most phylogeneticists accept that outgroup comparisons provide the best single guide to character polarity, thus in rejecting other inferences of polarity Collins-Rainboth & Buth (1990) adopt a sensible cautious approach that may have wrought some improvement over Lynch & Wake's (1975) character coding. However, this appears to have little practical effect. If Collins-Rainboth & Buth's (1990) interpretation of the data is amended by including all of Lynch & Wake's (1975) polarity assessments, Tree D remains the single MPT for the revised data, with no change in tree length.

A second major difference results from Lynch and Wake's (1975) experimentation with scaling their multistate characters. Collins-Rainboth & Buth (1990) weighted all characters equally, which is the commonest approach in numerical phylogenetics, but they did not discuss or attempt to justify their rejection of scaling. Farris (1990) has recently argued against scaling of phylogenetic data but cogent arguments for scaling have been presented by Thiele (1993). Use of scaling relates to the thorny issue of differential weighting of evidence, attitudes to which tend to be highly polarized. However, we would expect good phylogenetic data to support inferences that are insensitive to differences in potentially reasonable weighting strategies. Conversely, instability is indicative of limitations of the data, and the tentative nature of inferences based upon any preferred weighting scheme (Wilkinson and Benton, 1996). In practice, both Lynch & Wake's (1975) and Collins-Rainboth & Buth's (1990) interpretations of the data yield Tree A and Tree D respectively, irrespective of whether simple scaling or no scaling is employed. Thus, differences in scaling do not account for their different results. In contrast, bidirectional scaling, which is probably the least satisfactory of the methods used by Lynch & Wake (1975), particularly as it depends upon uncertain polarity assessments, does yield different trees.

Differences in the software used also do not fully explain the differences in Lynch & Wake's (1975) and Collins-Rainboth & Buth's (1990) results. Reanalysis using Lynch & Wake's (1975) interpretation of the data shows that the software they employed did not prevent them from correctly identifying the MPTs supported by their data. A single exception is their analysis using simple scaling with characters 7 and 10 omitted (treatment 8). Here the original analysis yielded only one of three MPTs.

A third major difference is in the method used to convert qualitative data into discrete characters. Many such methods have been proposed, though none are immune from criticism (Archie, 1985; Farris, 1990; Thiele, 1993). Collins-Rainboth & Buth (1990) presented no reasons for preferring their method over that of Lynch & Wake (1975), although their different results probably depend, at least in part, upon the different approaches. In practice, Collins-Rainboth & Buth's (1990) method fails to find any discrete character states for one of Lynch & Wake's (1975) characters, but finds additional character states for three others. Thus, neither approach can be categorized as the generally more conservative and, judged on their randomization test results, neither approach can claim to have yielded a more phylogenetically informative data set.

Other differences include Collins-Rainboth & Buth's (1990) minor change of coding of the septomaxillae and the omission of character 1 (standard length) from their reanalysis. This latter difference was also not discussed, but presumably reflects a desire to avoid non-independence (and thus overweighting) of this and other size related characters. In practice, this character is phylogenetically uninformative under parsimony and its omission can have no effect. In contrast, Collins-Rainboth & Buth (1990) showed no such concern for the possible independence of character 7, 8 and 10 discussed by Lynch & Wake (1975) and their recoding of character 9 ignored Lynch & Wake's (1978) observations and concerns.

In summary, the differences between Lynch & Wake's (1975) and Collins-Rainboth & Buth's (1990) results appear to depend primarily upon a few differences in their character coding, rather than, as Collins-Rainboth & Buth's (1990) believed, their use of more advanced software. Furthermore, neither of Lynch & Wake's (1975) or Collins-Rainboth & Buth's (1990) interpretations of the data are free from problems, and neither is demonstrably better than the other. The sensitivity of the results to character coding indicates which, if any, phylogenetic hypothesis is best supported by the observed variation is not clear cut.

Having found a single MPT supported by their revised data Collins-Rainboth & Buth (1990) addressed which of the four trees (Trees A-D) represent the best estimate of the phylogeny of Dendrotriton. Although they noted that the trees resulted from analyses that differed in coding of the data, scaling (weighting) and, in the case of Tree C, in method of analysis, they considered that the consistency indices for trees, based on their unscaled and revised character data, provided an appropriate comparative measure. Not surprisingly, consistency indices for Trees A-C were lower than those for Tree D (because they are not MPTs for these data), and on this basis, they proposed Tree D as the single best estimate of the phylogeny of Dendrotriton. However, and conversely, under Lynch & Wake's (1975) character coding and scaling Tree D is not most parsimonious. Thus, their preference for Tree D rests upon their preference for their character coding. Collins-Rainboth & Buth's (1990) enthusiasm for Tree D was not tempered by the observation that quite different trees result from minor differences in the treatment of the same underlying data or the observation that the differences in the consistency indices they considered are only marginal for Trees A and C.

Their confidence in Tree D was also strengthened by its insensitivity to the omission of the osteological characters. My reanalyses confirm this and also

demonstrate insensitivity to the omission of just the problematic character 9 (septomaxillae). Collins-Rainboth & Buth (1990) claimed to be following Lynch & Wake (1975) in performing their parallel analysis. However, as we have seen, Lynch & Wake (1975) omitted a different set of characters (7 and 10) because of their possible interdependence with character 8. If these characters are omitted from Collins-Rainboth & Buth's (1990) revised interpretation of the data (Fig. 3, treatment 18), there are dramatic changes in the MPTs supported by their data. Non-independence is a potentially serious problem because it can lead to overweighting of misleading phylogenetic signal and a false sense of confidence (Wilkinson, 1995). In as much as Lynch & Wake's (1975) concerns are reasonable, sensitivity of Collins-Rainboth & Buth's (1990) preferred tree to the omission of characters 7 and 10 must diminish confidence in that tree.

Collins-Rainboth & Buth's (1990) demonstration that Tree D is significantly shorter than the mean length of all trees appears to be intended to support their confidence in that tree, but this result is not surprising and has little phylogenetic significance. For example, the same significant difference obtains in the analyses of Lynch & Wake's (1975) data. What is more suggestive is the shape of the tree length distributions and g_1 , which, in their analyses and in all treatments in my reanalyses, suggests that the data do not contain strong phylogenetic signal.

Results from the renalyses using Lynch & Wake's (1975) interpretation of the data show a strong parallel to those for Collins-Rainboth & Buth's (1990) revised interpretation. In both analyses there is a single MPT supported by the full data, and insensitivity to the omission of some characters, but sensitivity to the omission of others, particularly those that may lack independence. In each case Bremer support for clades is low, differences in tree length between MPTs and one or more alternatives from among Trees A - D are marginal and g_1 statistics are unimpressive. The parallel suggests that Tree D is no better supported by Collins-Rainboth & Buth's (1990) interpretation of the data than are Trees A, B and C by some treatments using Lynch & Wake's (1975) interpretation.

A cautious view of the results of the reanalyses is that the sensitivity of phylogenetic inferences to variations in coding of data and analytical treatment, low Bremer support values and insignificant g_1 statistics imply that the data contains only weak (if any) phylogenetic signal, and that phylogenetic hypotheses based on these data should be viewed sceptically. This conclusion is fully supported by the results of the randomization tests. In none of the twenty treatments of the data can it be distinguished, based on tree length or compatibility, from randomly permuted data. That the null hypothesis that the data is phylogenetically uninformative cannot be rejected entails that hypotheses based on the data should be invested with no more confidence than a hypothesis based on random data or a randomly selected hypothesis, and that the available data are simply insufficient to resolve the phylogeny of *Dendrotriton*.

Collins-Rainboth & Buth (1990:960) commented that "Lynch and Wake (1975) predicted that further refinement of the knowledge of relationships within this group might come from the acquisition of new suites of characters, especially those generated using molecular technology. More data may strengthen our basis for estimation of relationships. However, advances in analytical methods may be as, or more important as technological advances in the generation of data." The present study reveals that Collins-Rainboth & Buth's (1990) results, and differences from those of Lynch & Wake's (1975), reflect more their modified interpretation of the data than they do advances in analytical methods and support Lynch & Wake's (1975) view that more data are needed.

Randomization tests are themselves a relatively recent addition to the analytical methods available in numerical phylogenetics, reflecting the increasing concern for the strengths and weaknesses of phylogenetic hypotheses and a shift away from more blinkered search for and acceptance of MPTs. As yet, they have not been widely applied, and some attitudes toward them are dismissive (e.g. Carpenter, 1992). However, this and other studies (e.g. Archie, 1989c; Faith, 1990) have identified real data that cannot be distinguished from randomly permuted data, with serious implications for the assessment of phylogenetic inferences based on the data.

The major obstacle to phylogenetic inference is the misleading evidence provided by homoplasy (Wilkinson, 1991). Interestingly, Wake (1991) has argued that homoplasy is rife among bolitoglossine salamanders. Three possible causes of this of this are (1) high rates of evolution in the variable characters of the group; (2) relatively rapid cladogenesis and thus short interior branch lengths such that most change occurs independently in terminal branches; and (3) a combination of 1 and 2. In as much as randomly permuted data are consistent with all three models of evolution, failure to discriminate between the data for Dendrotriton and random permutations of that data does not allow these models of evolution to be rejected, and supports Wake's (1991) view that there are high levels of homoplasy in bolitoglossine salamanders.

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NOTES ON THE FOOD HABITS OF *COLUBER HIPPOCREPIS NIGRESCENS* FROM PANTELLERIA ISLAND: A SNAKE THAT FEEDS ON BOTH CARRION AND LIVING PREY

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The food habits of Coluber hippocrepis nigrescens, a colubrid snake endemic to the island of Pantelleria in the southern Mediterranean Sea, were studied by means of both stomach contents and faecal analyses. Snakes preyed only on vertebrates, and most of the prey eaten were rodents (especially *Rattus norvegicus*). Ingested biomass per snake averaged 55.63 ± 48.94 g (range: 8 - 131 g), i.e. about 28.5 % of the average snake mass (195.18 ± 65.61 g; range: 39 - 277 g). Log prey mass and log predator mass were positively correlated. The prey mass to predator mass ratio was not significantly correlated with log predator mass. 36.36% of the prey found in *C. hippocrepis* stomachs was eaten by snakes when it was already carrion, as demonstrated by the presence of abundant flesh-fly larvae in rats regurgitated by snakes. This is a very unusual foraging mode for snakes, which are known as active predators feeding on live organisms. We hypothesize that this unusual foraging mode is an adaptive strategy depending on (1) low level of food availability and (2) high abundance of dead prey, on this arid Mediterranean island.

INTRODUCTION

Coluber hippocrepis is a colubrid snake widespread in north Africa and western Europe, and has scattered populations on some islands of the Italian Mediterranean Sea, i.e. Sardinia and Pantelleria (Bruno & Maugeri, 1990). In the former island this snake is very rare and endangered (Bruno & Hotz, 1976; Puddu, Viarengo & Erminio, 1988), whereas on Pantelleria it is widespread and relatively common (Cattaneo, 1985). The Pantelleria population has recently been described as an endemic subspecies, *C. hippocrepis nigrescens*, characterized by a high tendency towards dark colouration and melanism (Cattaneo, 1985).

Detailed data on the food habits of *C. hippocrepis hippocrepis* are available from Spain (Garzon, 1974; Vericad & Escarré, 1976; Pleguezuelos & Moreno, 1990). On the other hand, practically nothing is known about the Pantelleria population (Cattaneo, 1985), which should deserves attention by herpetologists due to its endemic status. This lack of knowledge is also serious because island populations of snakes may be very interesting to study. They may reveal remarkable lifehistory traits that are not normally observable in mainland populations, due to the rapid adaptive shifts possible in island taxa with small and discrete populations (Pendlebury, 1974; Schwaner, 1985; Shine, 1987; Capula, Rugiero & Luiselli, 1994).

The aim of this paper is to present preliminary information on the dietary habits of *C. hippocrepis nigrescens* from Pantelleria, focusing our attention on a very unusual foraging mode for snakes: feeding on prey corpses found in the field.

STUDY AREA AND METHODS

The field study was carried out in Pantelleria, an offshore volcanic island of the Mediterranean Sea (about 83 km² area) situated about 110 km SW of Sicily. The climate is Mediterranean-arid, with strong winds over much of the year. The vegetation is characterized by evergreen Mediterranean maquis, with very scarce arboreal cover. The snakes mainly inhabit grassy and bushy spots close to walls and stone piles. The surveyed area extended for about 95 ha. In the study area, C. hippocrepis is relatively widespread, although it cannot be considered common. Most of the adults encountered were about 110-140 cm long (total length), but larger individuals (all males) were occasionally found. The largest individual captured in the study area was 186 cm long (C. Bagnoli, personal communication), but there are records of specimens exceeding 190 cm (Cattaneo, 1985; A. Cattaneo, in verbis to C. Bagnoli). The melanistic condition was apparently more evident in large than in small individuals (Capula et al., unpublished observations).

We collected field data during three surveys conducted in the island of Pantelleria: June 1995, October 1995 and April 1996. A total of 32 field days (each aboutten hours long) were spent in the field. Moreover, additional data come from a survey by Mr Claudio Bagnoli (Rome), conducted in May, 1995.

When a snake was seen, it was captured by hand, sexed by analysing tail morphology, and body mass (to the nearest 0.1 g with an electronic balance) and SVL (snout-vent length, to the nearest 0.5 cm) were measured. Contrary to other studies (e.g. see Luiselli, Capula & Shine, 1996), we measured SVL rather than TL (total length) because several specimens had broken tails, possibly due to injuries caused by humans. Food items were collected by gentle pressing of the snake abdomen until regurgitation occurred, or by faecal analysis. Faeces were obtained only from snakes palpated in the field, and were not collected from the ground. Food items were identified to the lowest taxon possible. In the case of faeces, mammal hairs were easily recognized by medulla, cuticola and cross-section analyses, whereas saurian remains were identified by scale examination. Biomass of prey was measured only from stomach ingesta. In this case, we calculated the fresh mass of the prey as in Luiselli & Agrimi (1991). The snakes were also individually marked by "scale-clipping" to avoid multiple recounts of the same individuals. No snake was killed or injured during the course of this study.

RESULTS

Throughout the study period we captured a total of 36 snakes, including both captures (26 different specimens: 18 males and 8 females) and recaptures (10 recaptures of 9 individuals previously marked). The average capture rate was 1.12 snakes per day. The estimated population size (according to Lincoln's index in Seber, 1982, used as in Ineich, 1988) was 147.6 snakes, and the approximate density was 1.55 snakes per ha.

Handling of these snakes resulted in 28 prey samples, including those coming from both stomachs (n = 11) and faeces (n = 17). The summary of the dietary data is given in Table 1. The diet consisted only of vertebrates, and rodents constituted the main taxonomic category in the diet (71.43% of the total diet), followed

TABLE 1. Summary of the diet data obtained of *Coluber* hippocrepis nigrescens from the island of Pantelleria. Data come from analysis of both regurgitated ingesta and faecal pellets.

Prey type	N in faeces	<i>N</i> in stomachs	N total	%
Reptilia				
Podarcis sicula	4	3	7	25.0
Aves				
Passerine (unident	.) /	1	1	3.6
Mammalia				
Rattus norvegicus	6	5	11	39.3
Mus domesticus	1	2	3	10.7
Muridae (unident.)) 6	/	6	21.4
Total	17	11	28	100.0



FIG. 1. Biomass (g) of the prey items regurgitated by *Coluber hippocrepis nigrescens* individuals from the island of Pantelleria. For each prey category, the biomasses of the various items obtained from snake stomachs are shown.

by lizards (25%) and passerine birds (3.57%). Contingency table analysis revealed that rodents were preyed upon significantly more often than birds ($\chi^2 = 8.59$, df= 1, P = 0.0034), but not significantly more often than lizards ($\chi^2 = 3.13$, df = 1, P = 0.077). Rattus norvegicus was the most predated taxon, followed by Podarcis sicula and by unidentified rodents. In terms of ingested biomass, *R. norvegicus* was still the most important prey source for *C. hippocrepis nigrescens* (Fig. 1).

Unfortunately, our sample of snakes containing prey remains included only two juveniles, both of them with a lizard in the stomach. Thus, we cannot fully evaluate eventual ontogenetic shifts in the diet of this snake population.

Mean ingested biomass per snake was 55.63 ± 48.94 g (range: 8 - 131 g, n = 11), representing about 28.5 % of the average snake mass (195.18±65.61 g; range: 39 - 277 g, n = 11). The prey mass / predator mass ratio averaged 0.260±0.184 (range: 0.050 - 0.560, n = 11).

There was a significant positive correlation between log prey mass (as dependent variable) and log predator mass (r = 0.619, adjusted $r^2 = 0.315$, n = 11, intercept = -3.087, ANOVA: $F_{1,9} = 5.604$, P < 0.04; Fig. 2). However, the prey mass to predator mass ratio was not significantly correlated with log predator mass (r =0.244, n = 11, intercept = -3.087, ANOVA: $F_{1,9} =$ 0.570, P > 0.45), which means that the larger snakes did not tend to feed upon larger prey (relative to their own size) than the smaller snakes.

Four out of eleven (36.36%) prey found in *C. hippocrepis* stomachs were ingested by snakes when they were already carrion, i.e. they were not captured alive by the snakes. This was true for one ingested *Podarcis sicula* and three ingested *R. norvegicus*, weighing 131, 116 and 103 g respectively. In fact, examination of these disgorged rats revealed that their bodies were (1) full of flesh-fly larvae and (2) so damaged that they had clearly been killed by humans before being eaten by snakes. Examination of the lizard corpse



FIG. 2. Relationships between lg prey mass and lg predator mass in *Coluber hippocrepis nigrescens* from the island of Pantelleria. Regression statistics: r = 0.619, adjusted $r^2 =$ 0.315, n = 11, intercept = -3.087, ANOVA: $F_{1,9} = 5.604$, P <0.04.

revealed that it had clearly been squashed by cars before being ingested by the snake.

The unexpected necrophagous foraging mode of *C. hippocrepis* from Pantelleria was also confirmed by experiments on captive animals: four adult snakes, housed in outdoor enclosures, immediately accepted dead and partially putrefied mice (*Mus domesticus*) only four days after their removal from the natural environment, thus showing they were familiar with this unusual type of prey.

Ten out of eleven prey found in snake stomachs were ingested head first (*G* test with df = 1, P < 0.05). In this regard, *C. hippocrepis* from Pantelleria mirror Spanish conspecifics (Pleguezuelos & Moreno, 1990).

DISCUSSION

Data given here, although preliminary, reveal remarkable foraging patterns for the endemic C. *hippocrepis nigrescens* from the island of Pantelleria.

To begin with, the taxonomic dietary composition of the Pantelleria snakes was rather similar to that of populations of C. hippocrepis from elsewhere, e.g. Spain (Pleguezuelos & Moreno, 1990). Adult diet consisted mainly of rodents and, to a lesser extent, lizards. However, in comparison to the Spanish populations studied by Pleguezuelos & Moreno (1990), the Pantelleria population preyed on larger prey (given that the body sizes of the two snake populations were quite similar). With regard to prey size in relation to predator size, C. hippocrepis from Pantelleria are unusual compared not only to Spanish conspecifics but also to most of the colubrid species studied until now. In fact, whilst the average prey mass to predator mass ratio averaged 0.260±0.184 in the Pantelleria snakes studied here, this parameter averaged 0.18 in the various colubrid species from all over the world reviewed by Pough & Groves (1983), while the mean value for viperids was 0.36 (Pough & Groves, 1983). In this regard it is noteworthy that the prey mass: predator mass ratio of Pantelleria snakes is significantly higher (one-way ANOVA: P =(0.0306) than that of *Elaphe quatuorlineata* (mean = 0.125±0.123, see Capizzi & Luiselli, 1997), a large colubrid that suffocates prey before eating and that

could probably feed upon organisms larger (relatively to its own body size) than those normally available to non-venomous colubrids that eat live prey (e.g. *Coluber* species). Moreover, the mean prey mass: predator mass ratio of Pantelleria snakes was significantly higher (one-way ANOVA: P = 0.0023) than that (mean = 0.076 ± 0.053 , n = 13) of mainland *Coluber viridiflavus* (see Rugiero & Luiselli, 1995), a congeneric species characterized by similar (or even larger) body size. The same was true also for the mean size of the prey eaten, which was significantly larger (one-way ANOVA: P = 0.0059) in our island population of *C. hippocrepis* than in the mainland population of *C. viridiflavus* studied by Rugiero & Luiselli (1995) (mean = 13.09 ± 11.67 g, n = 13).

Why did *C. hippocrepis* from Pantelleria feed upon such unusually large prey? And, why did these snakes feed also upon carrion instead of having a "typical" snake diet based on living prey? We suggest that both questions could be answered with the argument that Pantelleria snakes developed such unusual traits in response to low level of live prey availability and high abundance of dead prey (chiefly rats killed by humans, cars and dogs), in this Mediterranean-arid island.

Indeed, it is in fact well-known that snakes inhabiting areas with low levels of food availability frequently try to capture and to ingest disproportionately larger prey (e.g. see Stergulc, 1983). On the other hand, foraging on carrion, although being an exceptionally rare event for free-living snakes, is a possible alternative strategy for these organisms, as demonstrated by the fact that many species (e.g. those belonging to the genera Vipera, Elaphe, Coluber, Coronella, Natrix, etc.) do easily accept dead prey when housed in captivity (Trutnau, 1981, 1982, and personal observations). Moreover, although rare, findings of free-ranging snakes scavenging on carcasses (also of large mammals such as wild pigs) have already been reported (e.g. see Heinrich & Studenroth, 1996; Torr & Richards, 1996). It is arguable that snakes consuming dead prey should probably be opportunistic predators that use chemical cues for foraging.

Foraging on corpses could also be important for permitting the Pantelleria snakes to feed upon disproportionaly large organisms in an island ecosystem with low levels of food availability. In fact, these "necrophagous" snakes could benefit from the fact that they have no difficulty associated with subduing large and vigorous prey. Moreover, it is likely that the advanced state of putrefaction of the ingested dead prey speed up digestion, thus reducing the digestive problems usually associated with eating prey of large volume. If this hypothesis is right, it is possible to conclude that foraging upon corpses might achieve for Pantelleria C. hippocrepis the same effect of speeding up digestion as the venom (with digestive properties) injected by vipers into the body cavity of their prey (e.g. cf. Bdolah, 1977).

Therefore, it is hypothesized that the alternative strategy of foraging on corpses could be widely found

in snakes, but that only in peculiar conditions do these predators adopt this alternative strategy. These peculiar conditions could thus be present on the island of Pantelleria.

Our study documents at least a partial shift in feeding habits of island *C. hippocrepis* in terms of (1) average prey size and (2) status of the prey (carrion versus living organisms). Previous studies on island populations of snakes also documented shifts in feeding habits, although the type of prey rather than the foraging mode was the trait subjected to shift. For instance, island *Notechis* prey more frequently on endothermic prey than those of the mainland (Shine, 1987), and similar shifts have also been observed in boids (Pendlebury, 1974) and pitvipers (Wharton, 1969). In all these cases the shifts were probably responses to differences in prey availability, the same being true in the case of *C. hippocrepis* of the island of Pantelleria.

As rightly pointed out by Shine (1987), it is, however very difficult to determine whether these shifts in feeding habits and foraging modes reflect geneticallybased adaptations or direct phenotypic responses to different food supplies.

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SHORT NOTES

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A REASSESSMENT OF HARDELLA ISOCLINA DUBOIS, 1908 (TESTUDINES: BATAGURIDAE) FROM THE TRINIL BEDS OF THE JAVAN PLEISTOCENE

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Hardella isoclina was described by Dubois (1908) from the Trinil Beds (Pleistocene) of Kedoeng Panas (= Kedung Panas), Java, Indonesia, also the source of the famous remains of Java man, Homo ("Pithecanthropus") erectus. The holotype and sole example of this fossil turtle species is at the Nationaal Natuurhistorisch Museum (previously Rijksmuseum van Natuurlijke Historie), Leiden and the original description of the new turtle fossil was brief and carried no illustrations. No reason was given for the assignment to the extant north Indian batagurid genus, Hardella, which is restricted to the flood plains of the rivers Indus, Ganges and Brahmaputra, in the northern parts of the Indian subcontinent (Smith, 1931; Das, 1995). Williams (1957) subsequently expanded the description, provided photographs and restorations of the fossil, diagnosis, and comparison with recent batagurids from tropical and subtropical Asia. The Trinil fossil was tentatively assigned to the genus Clemmys, then primarily a catchall taxon used for many small, narrow-headed emydids and batagurids with hexagonal neurals with short sides anteriorly. Williams (1957) commented that the knowledge of the skeletal morphology of Asian turtles that existed then did not permit a resolution to the problem of assigning a generic identity to the material from Trinil, but he indicated a close affinity of the fossil with Clemmys (now Mauremys) mutica. In the almost forty years that have elapsed since Williams' redescription, knowledge of the systematics and distributions of Asian turtles has increased to a point that permits us to grapple with the problem once again.

Williams (1957) compared Dubois' new species with a variety of south-east and east Asian turtle genera and species now assigned by some authorities (e.g. Gaffney, 1984; Gaffney & Meylan, 1988) to the family Bataguridae and assigned it (questionably) to the genus *Clemmys*, close to *C. mutica*, which is supported in a comparison with all known batagurid genera from southern and south-eastern Asia (see Table 1). The latter species is now included in the genus *Mauremys*, *Clemmys* having been restricted to certain North American species (McDowell, 1964). In Hirayama's (1984) scheme of classification, the Mauremys group of batagurids fall out from the rest of the members of the family. The Trinil fossil shows one important characteristic that differentiates Mauremys from Clemmys (sensu McDowell, 1964), the supracaudal scutes extending forward to cross the pygal/suprapygal seam, as well as several additional features that diagnose *Mauremys*, such as the slightly projecting rather than truncate gular region of the plastron, lack of a plastral hinge, and especially, hexagonal neurals (see McDowell, 1964). The ratio of the carapace width to carapace length (0.687), as well as the ratio of the width of the anterior plastral hindlobe at the level of the junction of the abdomino-femoral seam and the plastral margin to carapace length (0.436), are within the ranges reported by Iverson & McCord (1994) for adults and subadults of the east Asian species of Mauremys (0.665-0.781 and 0.425-0.517, respectively). The large size (estimated 308 mm carapace length) and the apparent absence of a plastral concavity suggest that the animal may have been a female: in a sample of recent species assigned to the genus Mauremys, Iverson & McCord (1994) found that females exceed males in carapace length, except in the Ryukyu (Iverson & McCord, 1994) and Taiwanese populations of M. mutica (Mao, 1971).

Cantor (1842) described Emys muticus (amended to *Emys mutica* by Gray, 1844) from Chusan (=Zhoushan Island), Zhejiang Province, China, and the species is now known to exhibit considerable geographic variation, resulting in a rich synonymy (see Iverson & McCord, 1989). McDowell (1964) allocated this taxon to the genus Mauremys (after a protracted life in Clemmys). Although juveniles of Mauremys mutica have been characterised in the literature as showing a tricarinate carapace (e.g. Pritchard, 1979; Ernst & Barbour, 1989), adult shells can be nearly smooth, with only a vestige of a vertebral keel. The illustrations of an adult Mauremys mutica (as Clemmys mutica) in Siebenrock (1909: PI. XVII-XVIII) are virtually identical to those in Williams (1957: PI. VII-VIII), except that in the former, vertebral I is widened anteriorly, vertebrals II-IV are more wide than long, the gular scales projecting more prominently, and the inguinal scales are relatively larger. The fossil turtle differs from Mauremys japonica (Temminck & Schlegel, 1835), in showing smooth marginals at the posterior of the carapace (serrated in the east Asian species) and the presence (vs absence) of a vertebral keel. It differs from the recently described Mauremys iversoni Pritchard & McCord, 1991, in showing an angular gular region (vs gular region rounded); absence (vs presence) of lateral keels; and the failure of the humero-pectoral seam to cross the entoplastron (vs humero-pectoral seam crosses entoplastron: see Fig. 2A in Pritchard & McCord, 1991). Given these minor differences, allocation of the Trinil fossil to the genus Mauremys seems appropriate.

Genus	Vertebral keels	Pleural keels	Humero- pectoral seam crosses entoplastron	Posterior margin of carapace strongly serrated	Plastral hinge	Plastral buttress strong or moderate	Neurals II & III short- sided anteriorly	Maximum carapace length
Batagur	+/-	_	-	_	_	+	+	600
Callagur	+	+/-	-	-	-	+	+	760
Chinemvs	+	+	+	-	-	+	+	120
Cuora	+/-	-	+	-	+	+	+	216
Cvclemvs	+	-	+	+	+	-	-	260
Geoclemvs	+	+	-	+/-	-	+	+	390
Geomvda	+	+	+	+	-	-	_	131
Hardella	+	+/-	-	-	-	+	+	610
Heosemys	+	+	+	+	-	-	_	242
Hieremys	-	-	+	-	-	-	-	450
Kachuga	+	-	-	+/-	-	+	+	580
Malayemys	+	+	-	-	-	+	-	210
Mauremys	+/-	+/-	+/-	-	-	+	+/-	195
Melanochelys	+	+	+/-	+/-	+/-	+/-	_	383
Morenia	-	-	-	-	-	-	+	210
Notochelys	+	-	+	-	+	+	+	320
Ocadia	-	-	+	-	-	-	+	271
Orlitia	+	1 4	-	+/-	-	-	+	760
Pyxidea	+	+	+	+	+	+	-	180
Siebenrockiella	+	+	+	+	-	_	+	170
Sacalia	+	-	+	-	-	-	+	176
"Hardella isoclina"	-	-	-	-	-	-	+	308

TABLE 1. Comparison of south and south-eastern batagurid genera with "*Hardella isoclina*" Dubois, 1908. + indicates a presence; - an absence. Measurements (in mm) of the carapace are the largest recorded for a species within the respective genus.

A subfossil pond turtle shell called Testudo anyangensis by Ping, 1930 has been reported from the ruins of Anyang Hsein in northern Honan, eastern China, which has been assigned to M. mutica by McDowell (1964), although Pritchard (1994) argued that it was conspecific with Ocadia sinensis. Living M. mutica are distributed over eastern China, including Taiwan and Hainan, the Ryukyu islands in southern Japan, and the Annam region of northern Vietnam, and have been introduced into Japan; M japonica is restricted to the islands of Honshu, Shikoku and Kyushu; and *M. iversoni* is known only from Fujian and possibly Guizhou Provinces of China (Iverson, 1992; Pritchard, 1979; Pritchard & McCord, 1991). In summary, no members of the genus have been recorded from the Sundaic region, including Java (see also Whitten & McCarthy, 1993). With a carapace length of 308 mm, the specimen from the Trinil bed is substantially larger than the largest Mauremys mutica on record (ca. "91/2 in" in Pritchard, 1979; and [as Annamemys annamensis] 19.5 cm in Ernst & Barbour, 1989). A larger size may not by itself conclusively demonstrate a specific status, as Pleistocene fossils of Geoclemys hamiltonii from Pakistan and northern India (Das, 1991), and Melanochelys tricarinata from northern India (Lydekker, 1889) are also known to be significantly larger than the maximum size reached by living representatives of the respective species.

As remarked by Williams (1957), the record introduces an apparently Chinese element into the Trinil fauna. *Mauremys isoclina* or its ancestors may have

entered the Sundas during the low waters of the Late Pleistocene glacials, when sea-levels dropped by as much as 120 m below present levels (Gascoyne et al., 1979), thereby connecting most of the Sundaic islands to what at present is continental Asia, permitting faunal exchange between the regions. Other remarkable Indo-Chinese and/or Asian mainland elements in the Sundaic fauna showing remarkable distributional disjunctions are on record. These include the sucker-mouthed loaches from hill stream environments that are currently assigned to Gyrinocheilidae, whose members are distributed in Indo-China, and while apparently absent in the Indo-Malayan region, reappear on north-western Borneo (see map in Berra, 1989: 67); the disjunct distribution shown by the discoglossid toads and ranid frogs of the genus Meristogenys, both of which have representatives on the Sundas, and further north, in south-eastern China (Inger, 1996); the batagurid turtle genus Geoemyda (fide McCord et al., 1995); anguinid lizard genus Ophisaurus (fide Inger, 1958), the colubrid snake genus Stoliczkaia (fide Smith, 1943), various species of mammals, including the tree shrew Dendrogale, the rodent Dremomys and the mustelid Melogale (Lord Medway, 1972); fossil representatives of hyaenids (Brongersma, 1937), and perhaps the distribution of Java Man himself (fide Hooijer, 1951). The widespread distribution of the aforementioned biota was doubtless facilitated by lowered sea levels that coincided with the Pleistocene glaciation. Some of the extant taxa among the present day Sundaic fauna are therefore of allochthonous origin, which have contributed to the overall high biological diversity of the Sundas.

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BODY TEMPERATURES OF THE MEXICAN LIZARD SCELOPORUS OCHOTERANAE FROM TWO POPULATIONS IN GUERRERO, MÉXICO

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Information on the thermal ecology of populations of the same species living in different habitats or elevations is needed if an understanding of the relative roles of physiology, thermal environments, and phylogeny in determining body temperatures in lizards is to be reached. Even among closely related species (e.g. congeners), there can be significant variation both in active body temperatures and in the extent to which body teminfluenced peratures are by environmental temperatures (e.g. Sceloporus; Lemos-Espinal, Smith, & Ballinger, in press). Body temperatures can even vary among populations of the same species, particularly when they occur in different habitats or at different elevations (Grant & Dunham, 1990; Van Damme, Bauwens, & Verheyen, 1990; Smith & Ballinger, 1994b; Lemos-Espinal & Ballinger, 1995).

The thermal ecology of the genus *Sceloporus* has been relatively well studied, and recent studies have expanded our knowledge to species from relatively unstudied regions of the genus' range (e.g. México) (see Lemos-Espinal, Smith, & Ballinger, in press for review). In this note, we report on an additional species of *Sceloporus* from México whose thermal biology has not been previously studied, *S. ochoteranae*. In addition, we compare two populations of *S. ochoteranae* from two distinct habitats and elevations.

One population was located in the Cañón del Zopilote north of Chilpancingo, Guerrero (600 m elevation), 14 km south of Mexcala, a small town on the Rio Balsas. The area is situated in arid tropical scrub (the most xeric portion of the Bosque Tropical Caducifolio of Rzedowski, 1988). Dominant vegetation includes the large cactus *Neobuxbaumia* sp., and trees such as *Bursera* spp. and *Acacia* sp. The area lies on the northern side of the Sierra Madre del Sur. There is a pronounced rainy season from late May until September at which time late afternoon and evening storms produce torrential rains. During the dry season the river is dry.

The other study population of *S. ochoteranae* was located in the Zitlala Mountains at km 1, rural road Acatlan-Zitlala, Guerrero (1250 m in elevation). This area is represented by the mountains located at the edge of a small cultivated valley. Some plant species observed in the area were *Pithecellobium dulce*, *Prosopis juliflora*, *Bursera* spp., *Acacia* spp., and *Mimosa* sp. The only crop observed in the mountains was *Zea mays*.

Lizards were captured by hand, noose, or rubber band. Body temperatures (T_b ; to the nearest 0.1°C) were obtained using quick reading cloacal thermometers. Care was taken to prevent temperature from being influenced by handling; thus all lizards requiring extensive capture effort were excluded from temperature recording. Body temperatures were measured only from active lizards (i.e. foraging or basking), and were taken throughout the day. Air temperature (T_a ; at 5 cm above substrate where lizard was first observed, using a shaded bulb to nearest 0.1°C) and substrate temperature (T_s ; on substrate where lizard was first observed, using a shaded bulb to nearest 0.1°C) were measured at the site of capture. We also measured snout-vent length (SVL; to nearest mm) using a ruler.

We used linear regression analyses to determine the relationships between T_b and T_a , T_s , and SVL. Comparisons of T_b between sexes and between populations were made using analysis of covariance to control for the effect of environmental temperatures on T_b . Either T_a or T_s were used as covariate in the analysis of covariance, depending on which variable explained the larger portion of the variance in T_b (i.e. which regression had the higher r^2 value).

Lizards in the Cañón del Zopilote population had an average SVL of $44.5 \pm 1.4 \text{ mm} (N = 33; \text{range} = 28 - 62 \text{ mm})$. The average T_b was $34.1 \pm 0.82^{\circ}\text{C}$ (N = 34); average T_a and T_s were $27.0 \pm 0.7^{\circ}\text{C}$ (N = 34) and $29.2 \pm 0.9^{\circ}\text{C}$ (N = 34), respectively. Body temperatures in this population increased with T_a $(N = 34, r^2 = 0.38, P < 0.0001; T_b = 16.0 + 0.67T_a)$. Body temperatures also increased with T_s , and T_s explained more variation in T_b than T_a $(N = 34, r^2 = 0.56, P < 0.0001; T_b = 14.2 + 0.68T_a)$. The T_b of an individual in this population was not related to its SVL $(N = 33, r^2 = 0.009, P = 0.59)$.

Males and females from the Cañón del Zopilote population did not differ significantly in T_b (ANCOVA with T_s as the covariate: $F_{1,31} = 0.80$, P = 0.38). Males had a mean T_b of 33.9 ± 1.2 °C (N = 19), whereas females had a mean T_b of 34.3 ± 1.1 °C (N = 15). The interaction between sex and T_s was not statistically significant, suggesting the slopes of the T_b on T_s regression did not differ between males and females and was therefore not included in the final ANCOVA model.

Lizards in the Zitlala Mountain population had an average SVL of $44.9 \pm 0.8 \text{ mm} (N = 57; \text{ range } 31 - 59 \text{ mm})$. The average T_{b} was $34.1 \pm 0.2^{\circ}\text{C}$ (N = 57), and the average T_{a} and T_{s} were $23.2 \pm 0.3^{\circ}\text{C}$ (N = 57) and

28.1 ± 0.5°C (N = 57), respectively. Body temperatures in this population increased with T_a (N = 57, r^2 = 0.19, P= 0.0007; T_b = 25.3 + 0.38 T_a); however, little of the variation in T_b was explained by T_a . Body temperature was also positively related to T_s in this population (N = 57, r^2 = 0.08, P = 0.04), but, as with T_a , very little vari-

ation was explained. The T_b of an individual in this population was not related to its SVL ($N = 57, r^2 = 0.03, P = 0.17$).

Males and females from the Zitlala Mountain population did not have significantly different T_b 's (ANCOVA with T_a as the covariate: $F_{1,54} = 0.03$, P = 0.87). Males had a mean T_b of $34.1 \pm 0.3^{\circ}$ C (N = 42), whereas females had a mean T_b of $34.0 \pm 0.5^{\circ}$ C (N = 15). The interaction between sex and T_a was not statistically significant, suggesting the slopes of the T_b on T_a regression did not differ between males and females and was therefore not included in the final ANCOVA model.

Individuals from the two populations appear to have very similar $T_{\rm b}$'s. Indeed the mean $T_{\rm b}$'s are identical: 34.1 ± 0.8 °C (N = 34) for the Cañón del Zopilote population and 34.1 \pm 0.2°C (N = 57) for the Zitlala Mountain population. However, when compared using ANCOVA with T_{a} as the covariate, the populations have statistically significantly different $T_{\rm b}$'s ($F_{1.88}$ = 11.1, P = 0.001). Comparing the least squares means of these population generated by the ANCOVA, the Cañón del Zopilote (32.7 \pm 0.5°C) has a lower mean $T_{\rm b}$ than the Zitlala Mountain population $(34.9 \pm 0.4^{\circ}C)$. The interaction between population and T_a was not statistically significant, suggesting the slopes of the $T_{\rm b}$ on T regression did not differ between the populations and was therefore not included in the final ANCOVA model.

The mean $T_{\rm b}$'s of the two populations of S. ochoteranae (c. 34°C) are well within the range of $T_{\rm b}$'s previously reported for lizards in the genus Sceloporus (28.9°C in S. variabilis, Benabib & Congdon, 1992; and 37.5°C in S. horridus, see Lemos-Espinal, Smith, & Ballinger, in press, for review). Body temperatures of individuals in the Cañón del Zopilote population of S. ochoteranae appear to be influenced to a greater extent by environmental temperatures than $T_{\rm b}$'s of individuals in the Zitlala Mountain population (i.e. had slope of $T_{\rm b}$ on $T_{\rm a}$ regression closer to one and larger r^2 values). Definite assignations of thermoconformity and thermoregulation could not be made because appropriate null models (see Hertz, 1992) could not be generated to directly assess the extent of thermoregulation in these populations of lizards.

In both populations of *S. ochoteranae*, males and females did not differ in mean T_b . Within the genus *Sceloporus*, whether or not the body temperatures of males and females are the same or different appears to depend on the species being considered. Indeed, males and females have been found to have the same T_b in several species (*S. gadoviae*, Lemos-Espinal, Smith, & Ballinger, in press; *S. grammicus*, Lemos-Espinal & Ballinger, 1995; S. jarrovi, Smith & Ballinger, 1994b; S. virgatus, Smith & Ballinger, 1994a), but males have also been found to have higher T_b 's than females (S. cyanogenys, Garrick, 1974; S. scalaris, Smith, Ballinger, & Congdon, 1993), and lower T_b 's than females (S. undulatus, Gillis, 1991).

The two populations of S. ochoteranae living in different habitats and different elevations appear to have the same absolute preferred $T_{\rm b}$ (c. 34°C); however, when the ambient temperature is considered, individuals in the Zitlala Mountain population appear to have a higher mean T_b than individuals in the Cañón del Zopilote population (i.e. least squares means are different). These results suggest that both populations physiologically "prefer" or are active at the same absolute $T_{\rm b}$, but that because of differences in the ambient conditions, Zitlala Mountain individuals must maintain a higher T, relative to ambient temperatures than do individuals in the Cañón del Zopilote. In other studies on populations of the same species living along an elevational gradient, or in different habitat types, the results concerning $T_{\rm b}$ differences are mixed. In some species, $T_{\rm b}$'s differ between populations at different altitudes or in different habitats (e.g., Grant & Dunham, 1990; Van Damme, Bauwens, Verheyen, 1990; Smith, Ballinger, & Congdon, 1993; Smith & Ballinger, 1994b), whereas in other species $T_{\rm b}$'s do not differ between populations at different altitudes or in different habitats despite differences in environmental temperatures (Adolph, 1990; Lemos-Espinal & Ballinger, 1995; Smith & Ballinger, 1995).

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BEHAVIOURAL OBSERVATIONS OF THE CHAMELEON CALUMMA OSHAUGHNESSYI OSHAUGHNESSYI IN MADAGASCAR

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One of the most important questions in behavioural research is how animals distribute themselves in space. The area regularly inhabited by an individual is known as its home range, the size and spacing of which depend on several factors, including food availability, habitat structure, social organization and population density (Stamps, 1977). A territory can be described as that part of the home range which is defended (Maher & Lott, 1995). Over the last thirty years, territoriality has been discussed primarily in terms of benefits to individual fitness through priority of access to limited resources and costs incurred through the exclusion of competitive conspecifics. More recent work, however, emphasizes the complexity of interaction between social and spatial factors, including possible benefits of living adjacent to conspecifics (Stamps, 1994).

Much of the information regarding territoriality in chameleons comes from studies of captive (Kästle, 1967; Bustard, 1965, 1966; de Vosjoli, 1979) and semicaptive (Parcher, 1974) individuals. Parcher (1974) attempted a field study of social behaviour in rain forest species during the wet season but due to the scarcity of interactions between individuals, resorted to artificial manipulation. These studies give detailed descriptions of territorial display between both males and females but provide little information about the characteristics of territories. Descriptions of territorial behaviour from the field are much rarer presumably due to the difficulty of observing these solitary and well camouflaged animals in dense habitats. Exceptions include studies of desert and grassland species (Burrage, 1973) and rain forest species during the dry season (Kauffmann, 1994). Burrage found both sexes of the terrestrial Chamaeleo namaquensis rigorously defended territories, but the arboreal species Bradypodium pumilum (as

C. pumilus) defended only roosting sites. Kauffmann reported that *Calumma brevicornis* and *C. oshaughnessyi ambreensis* maintained small, discrete home ranges (without the need for defence) often remaining faithful to a single roosting site on consecutive days.

Many species of lizards follow a cycle of daily activity, centred around behavioural thermoregulation. Basking takes place during the early morning and late afternoon to maximise heat uptake while the hottest hours of the day are spent in the shade or posturing at an angle which reduces heat absorption (Heatwole, 1976). In chameleons, thermoregulation has been studied primarily in species which are adapted to temperature extremes (Burrage, 1973; Reilly, 1982). These species were active throughout the day, maintaining remarkably constant body temperatures despite the fluctuating external environment, by using a range of physiological temperature controls such as colour lability, changes in lung volume and cardio-vascular adjustment (Burrage, 1973; Walton, Bennett & Losos, 1993).

As part of a wider study of chameleons in the eastern rain forest of Madagascar (Ranomafana National Park), we carried out some behavioural observations on *Calumma o. oshaughnessyi*. Our prime interests were spatial organization and daily activity patterns.

The study site (approximately 38x25 m) was situated in primary forest within the Ranomafana National Park. It encompassed a flat area of fairly uniform forest at an altitude of 1090 m. The canopy is closed and the understorey rich with lianes, palms and tree ferns. Research was carried out during the wet (breeding) season between 23.11.93 and 21.12.93. In order to investigate the spatial organization of this species, roosting sites of individuals were recorded on successive nights (after Kauffmann, 1994). The main path which ran through the study site was mapped, allowing the initial roosting sites (origins) of all chameleons to be measured in relation to the path. Chameleons were individually marked using typewriter correction fluid. The study site was searched regularly, new chameleons recorded and the roosting sites of known chameleons measured in relation to their origins. In addition, one of four focal chameleons was chosen for continuous observation during the day. This individual, located at night, was found roosting the next morning and watched from 06.00 to 1800 hr, in shifts of 1.5 hours. Observations were divided into 15 minute blocks within which a number of variables were recorded to determine the relationship between movement, foraging and time of day: total distance moved (estimated by eye); timing of a single 'pace' (defined as the complete cycle of movement by all four feet and measured once at the beginning of each period); number of strikes (tongue flicks) and miscellaneous observations. To obtain more detailed information about the use of space by individuals we measured the maximum height, minimum height, distance and bearing from the roost at each 1.5 hour change-over. Four adult chameleons (three fe-



FIG. 1. *C. o. oshaughnessyi* roosting sites (with occupation dates), during the behavioural study period.

males, F1-F3 and one male, M1) were watched over 13 days for a total of 114 hours.

Fig. 1 shows the initial locations of the four study chameleons and the positions of subsequent roosting sites. It can be seen that there is considerable spatial overlap but almost no temporal overlap. With the exception of 2.12.93 (when M1, F2 and F3 roosted close together) chameleons occupied the same area but at different times.

The maximum distance travelled from the roosting site, measured every 1.5 hours, was obtained for F2 and F3. Since the chameleons were watched continuously, these points can be joined together to give the two-dimensional area traversed during the day. Maximum and minimum heights were recorded for the same individuals and can be used to estimate the volume of space used by these individuals on a daily basis (Table 1).

No direct interactions between individuals occurred but short bouts of head jerking, consisting of two or



FIG. 2. *C. o. oshaughnessyi* behavioural observations. Individual averages were pooled to give the overall mean $(\pm SE)$ for all four chameleons. (a), hourly movement; (b), no. prey strikes (successful and unsuccessful) per hour; (c), pace per hour; (d), % frequency distribution of pace (taken only from days on which observations were completed from 06.01 to 18.00 hrs).

three sharp upwards movement of the head, were observed on 11 occasions for M1 and once for F3. We were unable to see the recipient, but head jerks have only been described as part of courtship, not agonistic, display (de Vosjoli, 1979; Parcher, 1974).

Mean hourly movement, mean number of strikes/ hour, mean pace/hour and the % frequency distribution of pace were calculated from our observation data (Fig. 2). Despite considerable individual variation, some general patterns emerged. After one or two hours of basking, activity increased during the morning although the distance travelled was only 2-3 m per hour. Movement decreased again towards late-afternoon before a final surge of activity at sunset, coinciding with the search for a roosting site. Foraging occurred throughout the day but peaked between 11.00 and 12.00 hrs. If a single pace took longer than the 15 minute recording block, the chameleon was considered to be stationary. 'No movement' was most frequently

 Chameleon	Date	Area (m ²)	Circumference (m)	Height (m)	Volume (m ³)
F1	1.12.93	3.81	13.12	3.0	11.43
F2	2.12.93	4.28	16.10	4.1	17.54
F3	20.12.93	2.79	9.27	5.5	14.78

TABLE 1. Use of space by individual chameleons.

recorded. For a moving chameleon, a single pace ranged from a few seconds to 13 mins, but was usually between one and three minutes. Movement during the afternoon was very slow whereas the return towards a roost at night was associated with an increase in speed.

The conclusions which can be drawn from our observations are clearly limited by the small sample size in this study. Nevertheless, use of space by the chameleons in this study was very different from that of two similar species (C. brevicornis and C. o. ambreensis) during the dry season (Kauffmann, 1994). Instead of maintaining small (mean size 29 m²) discrete home ranges, our study animals passed through the same area at different times, seldom returning to the same roosting site. Characteristics of the home range may vary with changes in the most sought-after resource. In seasonal habitats, an expansion of home range area is likely to occur during periods when there are high demands for food and/or mates (Rose, 1982). Burrage (1973) found that territories of male C. namaquensis increased during mate acquisition and those of females increased during egg-laying. The timing of reproduction in chameleons is varied (Lin & Nelson, 1981), but it is likely that breeding in most Malagasy species coincides with the rainy season. In this study, chameleons were occupying relatively large and overlapping home ranges, but the extent to which territories were held within these areas cannot be deduced. It is possible, for example, that the single male occupied a large territory through which the females travelled but much more information is needed on chameleon mating systems before this can be substantiated. Descriptions of chameleon agonistic display in the literature lead one to expect territorial behaviour but we saw no evidence of territorial or roosting site defence. It is interesting to note that territories are much more difficult to defend in visually complex habitats (Stamps, 1977). If territories are maintained, aggressive encounters are too rare to observe in the field (see Parcher, 1974).

Roosting site locations alone provide limited information about chameleon home ranges since the area visited during the day is not included. Kauffmann (unpub. data) found that during the dry season, the increase in home range area calculated using the outermost positions reached during the day was generally small (average increase for *C. o. ambreensis* = 12%). During this study chameleons followed a more irregular path between roosts indicating that home range size calculated using this method would have been severely underestimated. Nevertheless, roosting sites are obtained at night when chameleons are readily located and are useful in looking at relative differences between individual home ranges or within a population over time.

Home range size is particularly difficult to study in chameleons because of the three-dimensional nature of the arboreal habitat. Distribution within the vertical habitat may be an important means of resource partitioning within and between species of arboreal lizards. The difference between maximum and minimum heights recorded during the day gives a more accurate idea of the amount of space occupied by the chameleons. However, since chameleons sometimes ascended out of sight, these estimates of volume are probably conservative. F3 (20.12.93) moved further in the vertical plane than the horizontal, resulting in a larger overall space than F2 (1.12.93), although the area covered by F2 would have looked more extensive when presented on a two-dimensional map (Table 1). Improvements in canopy access techniques need to be applied to this area of study.

The pattern of daily activity shown here (Fig. 2) is consistent with behavioural thermoregulation to the extent that the cool early hours are spent basking and activity increases with increasing temperature. Maximum temperatures in this shady environment, however, are not sufficiently high to produce the midday inactive period typical of temperate lizards (Avery, 1993). Furcifer oustaleti exhibited similar activity patterns in warm secondary forest during the dry season in Madagascar (Kauffmann, unpub. data). This contrasts with the predominance of physiological thermoregulation described by Burrage (1973) and Reilly (1982) for desert and montane species. Perhaps in the relatively mild forest environment, the use of costly physiological thermoregulation is avoided. Further evidence for behavioural thermoregulation in rain forest species may come from observations during the dry/cold season in Madagascar (Kauffmann, 1994). In the early morning, chameleons travelled from their roost to a 'resting place' (either on a branch or under the leaf litter) where they maintained a long period of inactivity or torpor (up to 10 hours) before returning to roost. It is not certain, however, whether this behaviour resulted from low temperatures (daily mean 16°C) or food shortage. Reilly (1982) also noted differences between coldadapted chameleons which roosted in the interiors of shrubs/trees to avoid night time frost and warmeradapted species which roosted at the ends of branches in order to benefit from early morning sunshine. On many occasions, C. o. oshaughnessyi moved relatively large distances in the early evening just before arriving at their roosting site, suggesting such a preference for roost position.

Chameleons hunt by a combination of sit-and-wait predation and foraging while moving slowly through the habitat (Stamps, 1977; Burrage, 1973). This is confirmed by data from our study which shows that foraging occurred throughout the day, spanning periods with more and less movement (Fig. 2). However, the peak foraging time between 11.00 and 12.00 hrs was not accompanied by a large distance travelled, possibly due to the high activity of aerial prey. This, together with the most frequently recorded 'pace' of zero seconds, implies that sit-and-wait predation may be the predominant strategy in this species. There was no clear relationship between speed of movement and distance covered (Fig. 2) suggesting that chameleons travel with a combination of short bursts and slow, steady movements.

Some of the difficulties involved in the study of chameleon behaviour have been highlighted here. Without radio-tracking equipment, continuous observation is the only means of watching chameleons in the complex rain forest environment and may still result in the occasional focal individual disappearing from sight. Kauffmann (1994) used this method more successfully in the dry season, when chameleons were relatively inactive, but it is still a labour intensive and inefficient means of studying behaviour. More efficient sampling methods have been used successfully in open habitats: grassland and desert (Burrage, 1973); scrub (Lin and Nelson, 1981); montane scrub (Reilly, 1982). However, the use of radio-tracking is recommended for future studies, both for gaining information about chameleon spatial organization and to assist in finding and observing chameleons. Radio-tracking was used successfully in Kenyan scrub by Hebrard & Madsen (1984) and is currently being used to study the European chameleon in Spain (Loman, pers. comm.). However, even here there are a number of problems including high cost and poor signal reception in dense habitats. Nevertheless, with improved means of locating individuals in the field it will be possible to find out much more about the behaviour of these elusive animals

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ACTUAL AND OSTEOCHRONOLOGICAL ESTIMATED AGE OF NATTERJACK TOADS (*BUFO CALAMITA*)

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The estimation of individual ages in natural populations of amphibians is not a trivial undertaking (Halliday & Verrell, 1988). Intuitively, a mark-recapture method is the best approach to establish actual age by marking individuals at known ages, i.e. at metamorphosis, and recapturing them later when sexual maturity is attained and individuals migrate to ponds. However, this approach may involve two important costs. First, juveniles may exhibit very low survival rates (Smith, 1987; Semlitsch et al., 1988; Berven, 1990; Reading et al., 1991; Scott, 1994) and a large number of toadlets must therefore be marked. Second, if significant immigration rates occurred from unmarked populations, immigrants would be of indeterminate age compared with marked individuals of known age. An alternative method to estimate age in amphibians that overcomes these problems is the indirect assessment of age by studying the pattern of growth layers in the osseous tissues and counting the lines of arrested growth (LAGs) laid down in the midshaft diaphysis of long bones. This method is known as skeletochronology and in the past 15 years has become a widely used tool for ageing individual amphibians (see Smirina, 1994 for a review). This method, however, requires two conditions to be valid: (1) bone growth pattern must correlate with fairly constant chronological period; and (2) no significant variability in the genetic and/or environmental factors that control bone growth must exist within populations, thus allowing demographic comparisons both at intraand interpopulational level. Only a few investigations have dealt with both problems in natural populations (Smirina, 1972; Hemelaar & van Gelder, 1980; Gibbons & McCarthy, 1983; Frétey & Le Garff, 1996). All of these were made with recaptured animals that had achieved sexual maturity. LAGs were annual and this pattern did not show variability across individuals in these populations.

The present study was conducted in a natterjack toad (Bufo calamita) population located in Sierra Morena

(Córdoba province, southern Spain). As part of a longterm study, we marked around 2500 metamorphs by cutting the longest toe of the hind foot during the last two weeks of May 1992. Toadlets were seen around the ponds for nearly a month and then dispersed or aestivated because of the dry summer. In the first week of February 1995 rains filled the temporary ponds and a total of seven adult toads with clipped toes were recaptured. These animals allowed us to establish direct correspondence between an observed pattern of bone growth and the actual age of toads, which has never been reported in any amphibian species. We toe clipped the second outer toes of the right fore feet and these were preserved in 70% alcohol. We prepared bone sections for skeletochronological analysis following techniques published elsewhere (Hemelaar & van Gelder, 1980; Gibbons & McCarthy, 1983). From the toe selected, only the third phalange was used. This was washed in water for one hour, decalcified in 3% nitric acid for five hours and then rinsed in tap water overnight. Transverse sections of 15 µm were cut using a freezing microtome, stained with Ehrlich's haematoxylin for 15 min and rinsed in tap water for 30 min. Finally they were mounted in Aquamont and protected with a glass coverslip. The sections were examined with a Zeiss light microscope and photographed at a constant magnification. Age was determined by interpreting LAGs.

Five out of seven individuals clearly exhibited two distinct haematoxylinophilic lines of arrested growth. Individuals 7 and 16, however, exhibited an additional inner LAG (Fig. 1a). This line had probably been destroyed in the rest of toads due to bone remodelling at the periphery of the medullar cavity. Toads nos. 1 and 5 differed from this growth pattern showing two and one additional lines respectively (e.g. Fig. 1b).

These supplementary lines were more weakly expressed than the former and appeared alternated and more or less equidistant from the other, so it is improbable that they represented double-lines as reported in



FIG. 1. Haematoxylin-stained cross-sections of phalangic dyaphises in adult *Bufo calamita* metamorphosed in May 1992 and recaptured in February 1995. Numbers are designated lines of arrested growth (LAG). (A) Toad no. 7 female, 78 mm snout-urostyle length; (B) Toad no. 1 male, 81 mm snout-urostyle length. This individual showed two additional LAGs marked with arrows. Inner line one was lost probably due to the process of bone remodelling at the periphery of the medullar cavity.

other amphibian species (Francillon, 1979; Caetano & Castanet, 1993). The periodical or occasional character of these lines, and therefore their chronological value, cannot be stated here, given the small sample, but they very probably reveal the potential for intrapopulational variation in the number of arrested growth periods that natterjacks belonging to the same population may exhibit.

The number of growth rings may be correlated with the pattern of activity and growth that natterjacks exhibit in southern Spain. Contrary to northern populations, activity is minimal during the hot and dry summer, and this is presumably the period of arrested growth when LAGs are formed. Toads emerge after the autumn rains, but breeding is delayed until winter. A fraction of yearly adult growth, estimated from recaptured individuals, was achieved during the period of breeding (January-April), with remaining growth presumably occurring during autumn (Tejedo, 1992, Fig. 4). Whether juveniles exhibit this same growth pattern remains unknown, but juveniles were frequently observed near the breeding area in autumn and winter, and it seems unlikely that they were active during the dry summer. Therefore, the three LAGs observed may correspond to the aestivation periods of 1992 (first LAGs not resorbed in toads nos. 7 and 16, Fig. 1a), 1993 and 1994.

This paper has reported the first evidence of the correspondence between an observed pattern of bone growth and the actual age of toads. The fact that some individuals, in spite of the small sample, exhibited variation in the bone growth pattern by expressing light but distinct additional LAGs, casts doubt on the reliability of skeletochronology in estimating age in natural populations of amphibians in warm temperate climates. Complementary information about actual activity and retreat periods of the animals in natural populations together with information based on recaptured individuals of known real age is necessary to check and standardize ageing estimates based on bone growth.

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BOOK REVIEWS

Contributions to West Indian Herpetology. A Tribute to Albert Schwartz. Robert Powell and Robert W. Henderson (Eds.) (1996). 460 pp., 8 colour plates. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. \$60.00 (cloth).

This volume is the result of a two-day symposium held at the University of Georgia in 1994 in honour of Albert Schwartz, who died in 1992. In the introductory section, which also contains personal tributes, we learn that Schwartz's first publication on West Indian herpetology was in 1956 (a description of two new species from Cuba) and his last was in 1991 (the comprehensive and indispensable handbook for anyone interested in West Indian herpetology, "*Amphibians and Reptiles of the West Indies: descriptions, distribution and natural history*", co-authored by Robert Henderson). In the intervening 35 years he published another 193 papers on West Indian herpetology alone, a quite phenomenal output.

The reptiles and amphibians of the West Indies are diverse and fascinating, characterized by an extremely high degree of endemicity, and including two of the most speciose genera of vertebrates known (Anolis and Eleutherodactylus). It is hardly surprising that this unique natural laboratory has attracted research that has been at the forefront of the development of fields as diverse as evolution, ecology and behaviour. The next section of the book gives a brief history of the progress of West Indian herpetology and the people who have made significant contributions. It is nice to be able to put faces to the names on the papers that one has read many times! This section also includes a comprehensive annotated checklist of the herpetofauna (including meticulously researched complete literature citations for all 622 original species descriptions), followed by a series of colour plates introducing a range of different species. The photographs are largely of excellent quality and add value to the book.

The bulk of the book, however, consists of papers presented at the Symposium. These are arranged into three sections: the first is on evolution, systematics and biogeography, the second on ecology and behaviour, and the third on conservation. Finally, there is a small collection of abstracts of papers presented at the meeting but not submitted as full manuscripts. The papers have been written by 59 authors currently working on West Indian herpetology, from the United States, West Indies and Europe. Topics covered by the papers range from the broad-ranging (e.g. the origin of West Indian amphibians and reptiles) to the specific (e.g. the biology of the cave-dwelling Eleutherodactylus cooki). I was surprised to learn that despite many years of study, the herpetofauna of the West Indies is still comparatively poorly known: the rate of discovery of new species has by no means declined. In view of this fact, it is even more worrying that the number of species suffering major population declines has also increased, some of them reflecting global trends (e.g. the declining amphibian populations of Puerto Rico) while others reflect more local pressures (e.g. the Jamaican iguana).

All papers are abstracted in English, French and Spanish, which should serve to extend the usefulness of the contents. While the results of many of these studies have appeared in the primary literature, one normally tends to read only those which most directly impinge on one's own research, and so it is quite useful to be able so easily to contemplate the breadth of research currently being undertaken on West Indian herpetology. Considering the specialist nature of the book and its size, it seems reasonably priced. It will look good on the bookshelf, too. The dust jacket bears a striking and beautifully detailed hand-coloured drawing of Anolis chamaeleonides on the front. As if this wasn't enough, there is also a smaller reproduction of Tropidophis maculatus on the back, and an Anolis equestris adorns the title page. Each chapter is also headed (literally) by Cyclura cornuta, while the tailpiece is a diminutive Eleutherodactylus schwartzi.

Albert Schwartz was the single most significant contributor to our knowledge of the herpetofauna of the West Indies, and this beautifully presented volume is a fitting tribute to him.

Anita Malhotra University of Wales, Bangor

Die Strumpfbandnattern: Biologie, Verbreitung, Haltung. Frank Mutschmann. (1995). 172 pp. Die Neue Brehm-Bücherei, Westarp Wissenschaften. Magdeburg (paper).

The stated intention of this book is to provide an easily obtainable and accessible summary of pertinent facts on the snakes of the genus Thamnophis, particularly for herpetoculturists. The book starts with a slightly muddled consideration of the phylogenetic position of the garter snakes. This is followed by chapters on their anatomical characteristics, natural history, diseases and parasites, and captive husbandry. The systematic section consists of a key to the species, followed by a description and discussion of each species and subspecies, under the subheadings of size, description, distribution, natural history, and remarks, particularly on taxonomic status. The distribution of most species is mapped. Unfortunately, the same basic map of the whole of North and Central America is used for each species, resulting in far too little detail for species with restricted distributions. A synonymy of the species and subspecies of the genus is appended. The book is completed by a useful bibliography of about 300 references.

BOOK REVIEWS

No reference is given for the statement that the San Francisco garter snake (*T. sirtalis tetrataenia*) is no longer recognised as distinct from *T. s. infernalis*. This appears to be a misinterpretation of Boundy & Rossman (1995), who showed that the correct name for the San Francisco garter snake is *T. s. infernalis*, and assigned the populations previously called *T. s. infernalis* to *T. s. concinnus*. Considering the legal status of the San Francisco garter snake as an endangered taxon, it is important to emphasize that it is still recognized as a distinct subspecies, as before.

The line drawings are adequate but the monochrome photographs are generally of poor quality. Even many common species are only illustrated as preserved specimens. Two colour plates feature eight small photos each, of reasonably good quality. Given the commonness of many species of *Thamnophis* in captivity, it should have been possible to find much better photographs. In conclusion, Mutschmann's book provides a useful summary of the garter snakes, but without reaching a particularly outstanding level. German-speaking herpetoculturists and *Thamnophis* aficionados in general will want to own it, but there certainly remains room for a more comprehensive and scientific treatise of this genus.

REFERENCE

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