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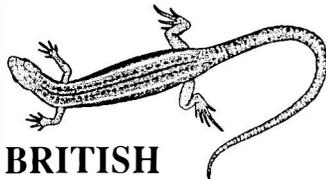
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FRONT COVER: Mallorcan midwife toad, *Alytes muletensis* (R. A. Griffiths)

THE WATER FROGS (ANURA: RANIDAE) OF TURKEY: A MORPHOMETRIC VIEW ON SYSTEMATICS

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The morphometric variation among 138 water frogs collected in Turkey at twelve localities extending from the Mediterranean coast in the south to the Black Sea coast was analysed using principal component and discriminant analyses. The water frog sample was heterogeneous and included two diagnosable morphs. Comparison with specimens from the type localities of *Rana bedriagae* (Damascus, Syria) and *R. ridibunda* (Atyrau, Kazakhstan) demonstrated that the most common water frog taxon in Turkey is *R. bedriagae*. The second morph was restricted to Ulubey, near Ordu, and was not conspecific with either *R. bedriagae* or *R. ridibunda*. It was, however, morphometrically closer to *R. bedriagae* than to *R. ridibunda*. As we were unable to locate an extant population of frogs which resembled the preserved sample from Ulubey, the taxonomic status of these morphometrically distinct water frogs remains unresolved. The large water frogs of the Anatolian Lakes District were indistinguishable from *R. bedriagae* in size-adjusted shape, but their maximum size exceeded that of *R. bedriagae* from all other localities by about 30 mm. We therefore provisionally refer to them as *R. bedriagae caralitana*. Reliable taxonomic recommendations require further information on independent character complexes such as advertisement calls and allozymes.

Key words: morphometry, *Rana bedriagae*, *R. bedriagae caralitana*, *R. ridibunda*, systematics

INTRODUCTION

Water frog systematics and biogeography of the Balkans and the Middle East have undergone considerable changes during the the past two decades (e.g. Schneider, Sofianidou & Kyriakopoulou-Sklavounou, 1984; Joermann, Baran & Schneider, 1988; Schneider & Sinsch, 1992; Sinsch & Schneider, 1996). At first the water frogs of this region were all referred to as *Rana ridibunda* PALLAS, 1771 (Boettger, 1888). Now, we know that the water frog fauna of this region (excluding the Mediterranean islands) comprises at least five species: *Rana balcanica*, *R. bedriagae*, *R. epeirotica*, *R. lessonae* and *R. ridibunda* and several types of hybrid (Schneider *et al.*, 1984; Schneider *et al.*, 1992; Schneider *et al.*, 1993; Sinsch & Schneider, 1996; Schneider & Sinsch, 1999). Nevertheless, our knowledge of the water frogs of Turkey and their biogeography remains limited because many regions are unexplored and taxonomic assignments are often uncertain.

Since the late 19th century Turkey was thought to be inhabited by the lake frog *Rana ridibunda* (Boettger, 1888; Werner, 1902, 1904; Bodenheimer, 1944; Mertens, 1952; Günther, 1991). This traditional view has been adopted in many faunistic, morphological and serological studies (Baran, 1981, 1984; Arıkan, 1983,

1990; Yılmaz, 1984; Atatür & Yılmaz, 1986; Baran *et al.*, 1992; Kaya, 1996; Kumlutaş, Tosunoğlu & Göçmen, 1999; Tok, 1999; Tok, Atatür & Ayaz, 2000). Consequently, reviews of the diversity of the herpetofauna of Turkey reiterate this point of view (Başoğlu & Özeti, 1973; Kasperek & Kasperek, 1990; Leviton *et al.*, 1992; Baran & Atatür, 1998).

The one-species concept was first modified by Arıkan (1988) who proposed that water frogs of the Lake Beyşehir region represent a new subspecies, *R. ridibunda caralitana*, because they differ from the other water frogs by the orange-coloured skin of wide parts of the venter and the legs and by their large snout-vent length. This striking coloration had already been observed by Kosswig (cf Bodenheimer, 1944). During the past decade several studies have focused on the geographical range of the new subspecies (Atatür, Arıkan & Mermer, 1989/90; Arıkan 1990; Arıkan *et al.*, 1994; Arıkan, Olgun, Çevik & Tok, 1998; Budak, Tok & Ayaz, 2000; Jdeidi, Bilgin & Kence, 2001). A recent mtDNA-based study of only 10 frogs from six localities suggests a remarkable degree of genetic differentiation among water frogs, but data are too scarce for reliable taxonomic conclusion (Plötner *et al.*, 2001).

Bioacoustic analyses of the advertisement calls of Palaearctic water frogs yielded results which did not agree with the assignment of water frogs in Turkey to *R. ridibunda*. Joermann, Baran & Schneider (1988) demonstrated that advertisement calls of water frogs from

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the Aegean coast (İzmir, Dalaman) had the same temporal structure as those from Israel (Nevo & Schneider, 1983), but differed from those of water frogs from the Balkans. Extensive comparative studies of the vocalizations of water frogs in the Middle East and adjacent regions including the type localities of *R. ridibunda* at Atyrau (Kazakhstan) and *R. bedriagae* at Damascus (Syria) finally provided evidence that Israel, Syria, the Nile delta of Egypt and the studied regions of Turkey are inhabited by the Levantine frog, *R. bedriagae* (Akef & Schneider, 1989; Schneider & Sinsch, 1992, 1999; Schneider, 1997a,b, 1999). Further bioacoustic surveys extended the geographical range of *R. bedriagae* in Turkey (Schneider & Sinsch, 2001).

The species status of *R. bedriagae* and the assessment of its geographical range is not only based on bioacoustics. Electrophoretic studies on allozymes (Nevo & Filippucci, 1988; Sinsch & Eblenkamp, 1994; Sofianidou, Schneider & Sinsch, 1994) and comparative morphometrics (Sinsch & Schneider, 1999) provided further characters which distinguish *R. bedriagae* from *R. ridibunda*. In particular, the morphometric study extended its range to many more sites in Syria, Jordan and Turkey. The combined evidence suggests that *R. bedriagae* is widely distributed in the Middle East (Sinsch & Schneider, 1999).

The morphometric evidence of the presence of *R. bedriagae* at Alanya and Lake Beyşehir (Turkey) is inconsistent with the conclusions drawn from other morphological studies. The nominate form of *R. ridibunda* is claimed to inhabit the northern Aegean islands as well as those of the Sea of Marmara and the Black Sea (Baran 1981), Turkish Thrace (Yılmaz, 1984), the southern Lakes district (Atatür, Arıkan & Mermer, 1989/1990), four more regions of Turkey (Atatür, 1990), the Datça peninsula (Tok, 1999) and Dalaman on the Aegean coast (Tok, Atatür & Ayaz, 2000). Some of the contradicting taxonomic assignments may be due to the fact that the studied water frogs have not been compared with animals from the type localities of *R. bedriagae* and *R. ridibunda*. However, data may also indicate that *R. bedriagae* is not the only water frog species of Turkey. Initial bioacoustic data suggest that *R. ridibunda* occurs in the Kızılırmak River at Gülşehir, central Turkey (Schneider & Sinsch, 1999). *R. ridibunda* is likely to be present in regions in which climate is unfavourable for *R. bedriagae*, such as Turkish Thrace and the Black Sea coast (Schneider & Sinsch, 2001). Consequently, we used morphometric data on water frogs collected at twelve localities extending from the Mediterranean coast in the south across the country to the Black Sea coast to test the hypothesis that more than one water frog species inhabits Turkey. Principal component and discriminant analyses were applied to detect and to quantify local features of the populations studied in Turkey. To enable a reliable taxonomic assignment to either *R. bedriagae* or *R. ridibunda*, we included reference populations (also

from the corresponding type localities) of both species in the analysis.

MATERIAL AND METHODS

Water frogs ($n=138$) were collected in Turkey at the following localities (Fig. 1): (1) Alanya (Antalya), $n=22$; (2) Lake Beyşehir (Konya), $n=32$, type locality of *Rana ridibunda caralitana* Arıkan, 1988; (3) Yakaköy near Dinar (Afyon), $n=14$; (4) Lake Işıklı at Beydilli (Denizli), $n=9$; (5) Lake Eber at Çayırpınar (Afyon), $n=6$; (6) Çifteler (Eskişehir), $n=5$; (7) Balçıkhisar, ca. 30 km north of Çifteler (Eskişehir), $n=12$; (8) Lake İznik at Çakırca (Bursa), $n=8$; (9) Karasu (Adapazarı), $n=5$; (10) Kızılırmak at Bafra (Samsun), $n=5$; (11) Ulubey (Ordu), $n=16$; (12) Turna Suyu (Ordu), $n=4$. Ten external morphological characters were measured with calipers to the nearest 0.1 mm: (1) snout-vent length (SVL); (2) callus internus length (CIL); (3) digitus primus length (DPL); (4) femur length (FEMUR); (5) tibia length (TIBIA); (6) foot length (FOOT); (7) head width at eye position (HEADeye); (8) maximal head width (HEADmax); (9) snout-eye distance (SNOUT-EYE); (10) tympanum diameter (TYM). Comparisons of these water frogs with species that are known to inhabit neighbouring states were made by comparing the morphometrics with corresponding measurements of reference samples of *Rana bedriagae* CAMERANO, 1882 ($n=94$) and of *R. ridibunda* PALLAS, 1771 ($n=55$) which were shown to be homogeneous in Sinsch & Schneider (1999). The reference samples included individuals from the type locality of *R. bedriagae* at Damascus, Syria, and from the type locality of *R. ridibunda* at Atyrau, Kazakhstan. Localities, numbers and sex of specimens and their assignment to museum collections are summarized in Appendix 1.

All measurements were taken by one of us (H. Schneider) from either preserved frogs or from live frogs that were captured and released at the capture site. We tested data (principal component scores, ratios) for a potential bias between preserved and living material in

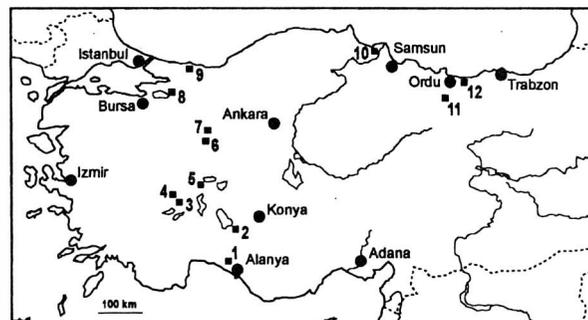


FIG. 1. Map of localities. 1 Alanya (Antalya); 2 Lake Beyşehir (Konya, type locality of *Rana ridibunda caralitana* Arıkan, 1988); 3 Yakaköy, Dinar (Afyon); 4 Lake Işıklı at Beydilli (Afyon); 5 Lake Eber at Çayırpınar (Afyon); 6 Çifteler (Eskişehir); 7 Balçıkhisar, ca. 30 km north of Çifteler (Eskişehir); 8 Lake İznik at Çakırca (Bursa); 9 Karasu (Adapazarı); 10 Kızılırmak at Bafra (Samsun); 11 Ulubey (Ordu); 12 Turna Suyu (Ordu).

TABLE 1. Principal component analysis of the data set on 138 water frogs from Turkey consisting of ten log₁₀-transformed, standardized morphometric variables. The association (=component weights) of single variables to the derived three principal components representing a total of 94.4% of total variance is presented.

	PC 1	PC 2	PC 3
Eigenvalue	8.87	0.32	0.25
Variable:			
SVL	0.331	0.044	-0.002
CIL	0.295	0.236	-0.894
DPL	0.300	-0.549	-0.140
FEMUR	0.318	-0.089	0.195
TIBIA	0.328	-0.080	0.126
FOOT	0.319	-0.223	0.153
HEADeye	0.327	-0.078	0.114
HEADmax	0.328	0.080	-0.006
SNOUT-EYE	0.320	-0.044	0.112
TYMP	0.292	0.750	0.280

the sample collected at Lake Beşyehir, but the observed deviations with respect to size- or shape-related measures did not reach statistical significance. We therefore assumed that preservation did not affect the parameters used for this analysis and pooled data irrespective of preservation state.

Before applying multivariate statistics all morphometric distances were log₁₀-transformed. As sexual dimorphism was limited to differences in size

(Table 7), we pooled single measurements into three data sets irrespective of sex: (1) frogs only from Turkey; (2) frogs from Turkey + *R. bedriagae*; (3) frogs from Turkey + *R. ridibunda*. Each data set was subjected to principal component analysis to explore the morphometric variability independent of taxonomic assignment, i.e. to test for homogeneity of the sample and to reduce the information to statistically unrelated factors. The first principal component (PC1) of morphometric data generally describes differences in size, but size effects may be present in subsequent principal components representing shape (Humphries *et al.*, 1981). The second, third and subsequent components are related to aspects of shape. Slopes and intercepts of regression lines describing the size-PC1 relationship were compared using ANOVA. Discriminant analysis was applied to maximize the differences among predefined groups, i.e. water frogs from Turkey, *R. bedriagae* and *R. ridibunda*. Using the rate of correct classification, we obtained a quantitative measure of the morphological differentiation among the groups/species (Schneider & Sinsch 1992).

RESULTS

MORPHOMETRIC VARIATION AMONG WATER FROGS FROM TWELVE LOCALITIES IN TURKEY

Most of the morphological variability (88.7%) within the data set of 138 water frogs was caused by variation in size, i.e. PC1 (Table 1, Fig. 2A). The allometric relationship between PC1 and snout-vent length did not differ among frogs from different localities. However, maximum size was largest in the sample from Lake Beşyehir (122.4 mm), intermediate in that from Ulubey (103.9 mm), and smallest in the other ten samples (72.2–88.4 mm). With respect to shape, the frogs from Ulubey varied considerably from all others (including those from the neighbouring localities Kızıllırmak at Bafra and Turna Suyu) with respect to their scores on PC2, which accounted for 3.2% of total variation (Fig. 2B). PC2 was strongly loaded by the variable tympanum diameter (Fig. 3C), but also by the variables representing foot morphology (DPL, FOOT, CIL; Table 1). Thus, principal component analysis suggested that the data set tested was not homogeneous with respect to shape-related variables. The discriminant analysis lent further support to the supposed sample heterogeneity: five significant discriminant functions were derived (Table 2). Successful classification of individuals to their actual population exceeded the rate of random assignment (8.33%) by a factor of four to ten, suggesting the existence of particular local characteristics. In four populations (Lake Eber at Çayirpinar, Çifteler, Karasu and Ulubey) classification success was 80% or more (Table 2C). Specifically, the scores of the Ulubey frogs showed little overlap with the distributions of those from the other regions. In contrast, the frogs from Lake Beşyehir only yielded a classification success of 44% (>5 times the probability of random assignment).

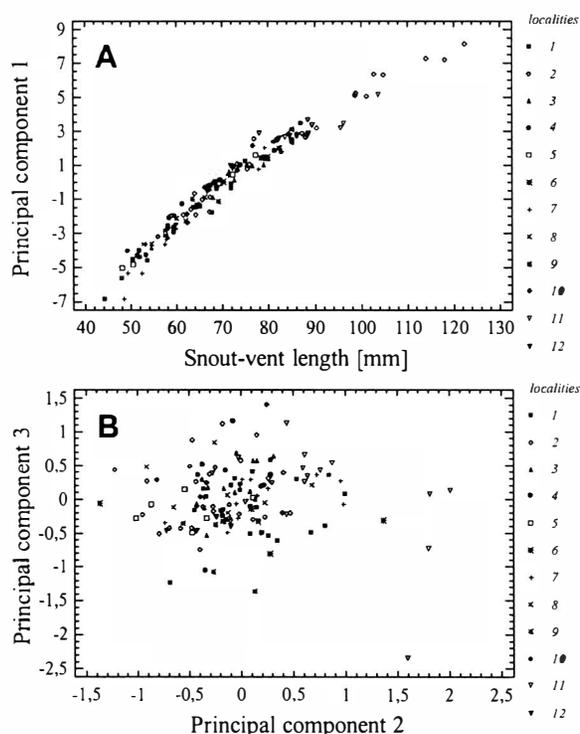


FIG. 2. Morphometric variation among the water frogs from 12 localities (see Fig. 1) in Turkey. (A) Size-related variation; (B) Shape-related variation. Details on the principal component analysis are given in Table 1.

TABLE 2. Significant discriminant functions based on ten \log_{10} -transformed morphometric variables to distinguish among water frogs from 12 localities in Turkey (see Fig. 1). In (C) the values shown are actual (1st row) and predicted (2nd row) localities.

<i>(A) Statistical significance:</i>							
Discriminant function	Eigenvalue	Relative Percentage	Canonical Correlation	Wilks Lambda	χ^2	df	<i>P</i>
1	1.61	41.7	0.786	0.063	348.7	110	<<0.0001
2	0.96	24.8	0.699	0.164	227.6	90	<<0.0001
3	0.50	13.0	0.578	0.322	142.9	72	<<0.0001
4	0.28	7.2	0.467	0.483	91.6	56	0.0019
5	0.20	5.1	0.406	0.618	60.6	42	0.0315

<i>(B) Unstandardized coefficients of the significant discriminant functions:</i>					
	Discriminant functions				
	1	2	3	4	5
SVL	0.09	-34.77	18.46	21.67	-17.11
CIL	10.35	1.63	-2.33	-3.26	-12.38
DPL	0.40	11.36	8.14	4.96	-3.72
FEMUR	-24.20	-18.87	9.51	-15.24	-2.73
TIBIA	8.99	10.99	-33.32	36.15	29.67
FOOT	11.81	14.79	4.22	-10.86	-16.67
HEADeye	-8.74	3.68	26.86	-6.69	2.36
HEADmax	16.68	3.22	-27.49	-30.56	18.18
SNOUT-EYE	-13.39	20.86	0.89	0.28	11.30
TYM	7.83	-11.40	3.28	4.52	-2.38
CONSTANT	-6.90	15.46	-12.02	-11.26	-10.80

<i>(C) Classification success:</i>												
	1	2	3	4	5	6	7	8	9	10	11	12
1	15 68.2%	-	-	-	-	4 18.2%	2 9.1%	-	1 4.6%	-	-	-
2	1 3.1%	14 43.8%	4	5 12.5%	4 15.6%	-	-	2	1 6.3%	1 3.1%	-	-
3	1 7.1%	-	10 71.4%	1	1 7.1%	-	-	-	1 7.1%	-	-	-
4	-	2 22.2%	1 11.1%	5 55.6%	1	-	-	-	-	-	-	-
5	1 16.7%	-	-	-	5 83.3%	-	-	-	-	-	-	-
6	-	-	-	-	-	4 80.0%	-	-	-	-	-	1 20.0%
7	3 25.0%	1 8.3%	-	-	-	2 16.7%	4 33.3%	1 8.3%	-	-	1 8.3%	-
8	-	1 12.5%	-	-	-	-	-	6 75.0%	-	-	-	1 12.5%
9	-	-	-	-	1 20.0%	-	-	-	4 80.0%	-	-	-
10	-	-	-	2 40.0%	-	-	-	-	-	2 40.0%	-	1 20.0%
11	-	2 12.5%	-	1 6.3%	-	-	-	-	-	-	13 81.2%	-
12	-	-	-	1 25.0%	1 25.0%	-	-	-	-	-	-	2 50.0%

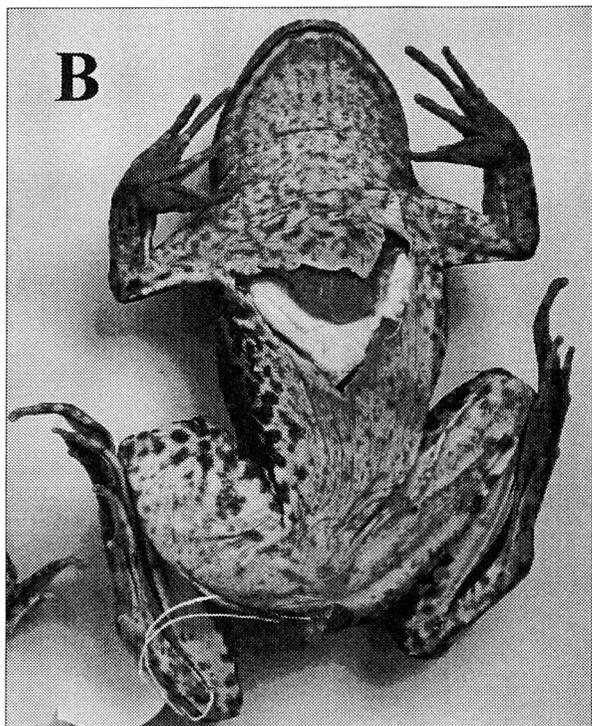
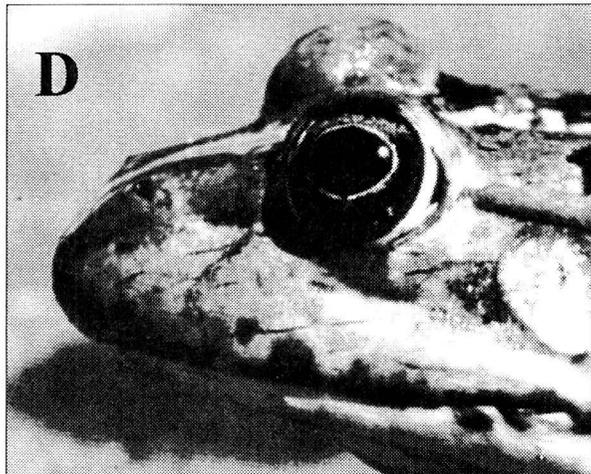
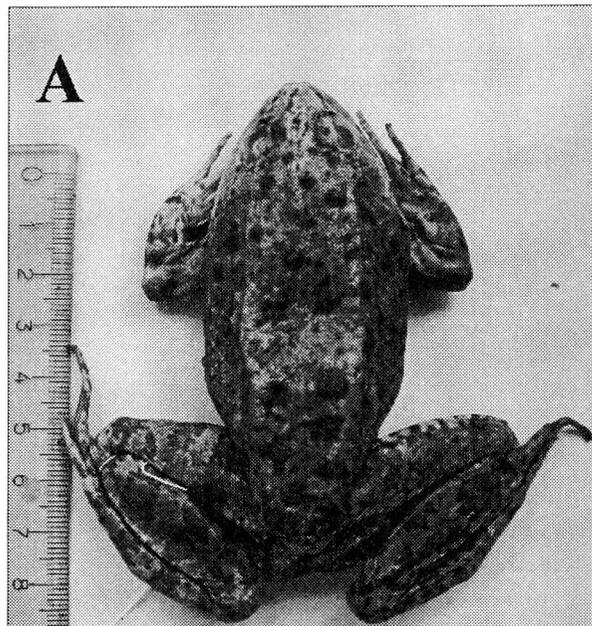


FIG. 3. Female water frog collected at Ulubey, Turkey (A-C), *Rana bedriagae* from Hadera, Israel (D) and *Rana ridibunda* from Valtos, Greece (E).

TABLE 3. Principal component analysis of the data set on 138 lake frogs from Turkey and 94 *Rana bedriagae* from Syria, Jordan, and Israel consisting of ten log₁₀-transformed, standardized morphometric variables. The association (=component weights) of single variables to the three principal components representing a total of 93.9% of total variance is presented.

	PC 1	PC 2	PC 3
Eigenvalue	8.72	0.41	0.25
Variable:			
SVL	0.332	-0.084	0.093
CIL	0.299	0.127	-0.822
DPL	0.308	0.007	-0.321
FEMUR	0.314	-0.357	0.199
TIBIA	0.332	-0.034	0.093
FOOT	0.323	0.032	0.048
HEADeye	0.327	-0.225	0.112
HEADmax	0.331	0.010	0.022
SNOUT-EYE	0.317	-0.155	0.256
TYMP	0.274	0.879	0.290

TABLE 4. Significant discriminant functions based on ten log₁₀-transformed morphometric variables to distinguish among water frogs from 12 localities in Turkey and *Rana bedriagae*. In (C) the values shown are actual (1st row) and predicted (2nd row) localities.

(A) Statistical significance:							
Discriminant Function	Eigenvalue	Relative Percentage	Canonical Correlation	Wilks Lambda	χ ²	df	P
1	1.44	51.0	0.769	0.124	458.7	120	<<0.00001
2	0.59	20.9	0.610	0.302	262.7	99	<<0.00001
3	0.27	9.5	0.460	0.481	160.8	80	<<0.00001
4	0.17	5.9	0.378	0.610	108.6	63	0.0003
5	0.14	4.8	0.346	0.711	74.8	48	0.0080

(B) Unstandardized coefficients of the discriminant functions:					
	Discriminant functions				
	1	2	3	4	5
SVL	12.18	22.52	-22.63	6.00	20.55
CIL	5.25	1.78	3.56	8.62	4.07
DPL	1.36	-15.72	-9.80	3.68	0.59
FEMUR	-16.36	13.07	-4.92	11.02	-11.75
TIBIA	-4.04	4.45	17.26	-51.13	28.07
FOOT	12.86	-20.03	3.03	18.37	0.60
HEADeye	-3.86	-9.64	-23.42	5.98	-3.90
HEADmax	13.67	3.13	42.03	-4.84	-25.33
SNOUT-EYE	-19.64	-13.13	-0.71	-10.48	-16.91
TYM	8.57	12.08	-11.43	4.43	-1.54
CONSTANT	-15.99	-6.71	8.46	19.44	-7.59

(C) Classification success:													
	1	2	3	4	5	6	7	8	9	10	11	12	<i>R. bedriagae</i>
1	12 (54.6%)	-	1 (4.6)	-	-	3 (13.6%)	2 (9.1%)	-	-	-	-	1 (4.6%)	3 (13.6%)
2	-	15 (46.9%)	5 (15.6%)	5 (15.6%)	4 (12.5%)	3 (9.4%)	-	-	1 (3.1%)	1 (3.1%)	1 (3.1%)	-	2 (6.3%)
3	-	-	9 (64.3%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	-	-	1 (7.1%)	-	-	-	1 (7.1%)
4	-	3 (33.3%)	2 (22.2%)	3 (33.3%)	1 (11.1%)	-	-	-	-	-	-	-	-
5	-	-	-	-	5 (83.3%)	-	-	-	-	-	-	-	1 (16.7%)
6	-	-	-	-	-	4 (80.0%)	-	-	-	-	-	1 (20.0%)	-
7	3 (25.0%)	1 (8.3%)	-	-	-	2 (16.7%)	3 (25.0%)	1 (8.3%)	-	-	1 (8.3%)	-	1 (8.3%)
8	-	1 (12.5%)	-	-	1 (12.5%)	-	1 (12.5%)	5 (62.5%)	-	-	-	-	-
9	-	-	-	-	1 (20.0%)	-	-	-	3 (60.0%)	-	-	-	1 (20.0%)
10	-	-	-	1 (20.0%)	2 (40.0%)	-	-	-	-	2 (40.0%)	-	-	-
11	-	1 (6.3%)	-	-	-	-	-	-	-	-	15 (93.8%)	-	-
12	-	-	-	1 (25.0%)	1 (25.0%)	-	-	-	-	-	-	2 (50.0%)	-
<i>R. bedriagae</i>	15 (16.0%)	1 (1.1%)	2 (2.1%)	5 (5.3%)	4 (4.3%)	5 (5.3%)	9 (9.6%)	7 (7.5%)	5 (5.3%)	-	-	8 (8.5%)	33 (35.1%)

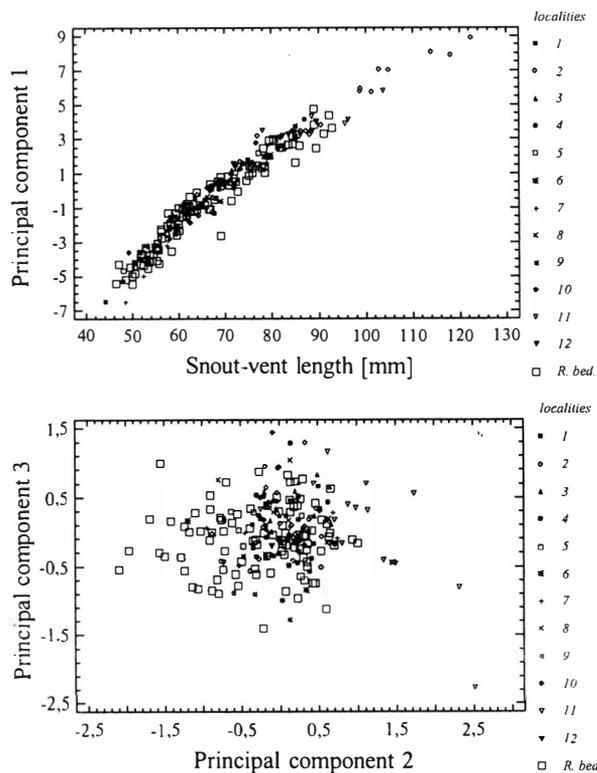


FIG. 4. Morphometric variation among the water frogs collected at 12 localities in Turkey and *Rana bedriagae* from Syria, Jordan and Israel. (A) Size-related variation; (B) Shape-related variation. Details on the principal component analysis are given in Table 3.

TAXONOMIC IDENTIFICATION OF WATER FROGS IN TURKEY

Initially, we compared the features of the frogs from Turkey with those of *R. bedriagae* that inhabit Syria, Jordan, Israel and Egypt (Table 3). The size-related PC1 accounted for 87.2% of total variance of the pooled data set (Fig. 4A). The comparison of regression lines demonstrated that the relationship SVL/PC1 did not differ between the frogs from Turkey and *R. bedriagae* from outside Turkey (ANOVA: intercepts $P > 0.05$; slopes $P > 0.05$). The shape-related PC2 and PC3 (accounting for 4.1% and 2.5% of total variance,

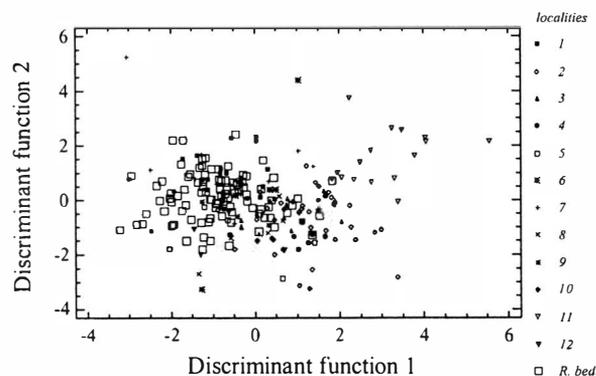


FIG. 5. Distinction among water frogs collected at 12 localities in Turkey and *Rana bedriagae* from Syria, Jordan and Israel by discriminant functions. Details on the discriminant analysis are given in Table 4.

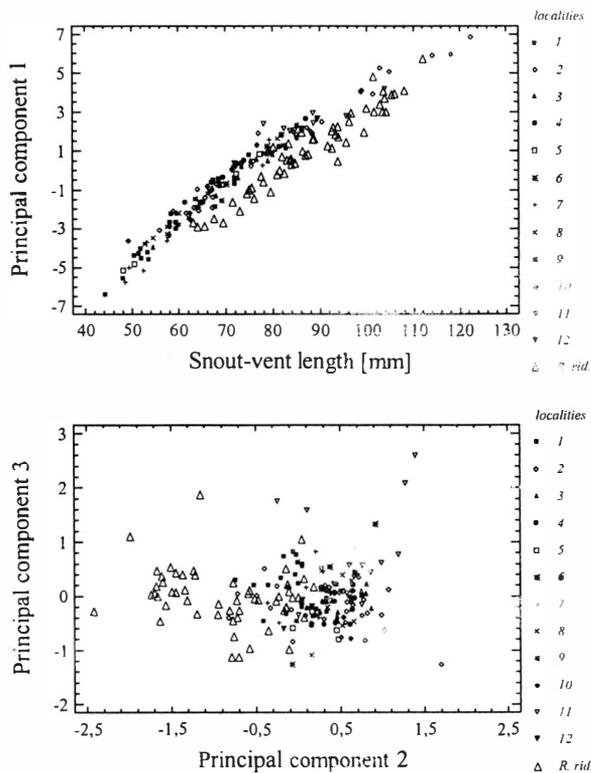


FIG. 6. Morphometric variation among the water frogs collected at 12 localities in Turkey and *Rana ridibunda* from Khazakistan, Armenia and Greece. (A) Size-related variation; (B) Shape-related variation. Details on the principal component analysis are given in Table 5.

respectively) yielded a complete overlap of scores between *R. bedriagae* and all frogs from Turkey, except for those from Ulubey (Fig. 4B). Again, PC2 mainly represented tympanum features. Discriminant analysis yielded five significant discriminant functions (Table 4). The classification success of the individuals into their actual population again exceeded the rate of random assignment (7.69%) by 3.3-12.2 times, but decreased in most samples due to the presence of *R. bedriagae*, as the morphological variability of this large sample covered most of the variation range of populations in Turkey (Table 4C). The only notable exceptions to this rule were the frogs from Ulubey, which

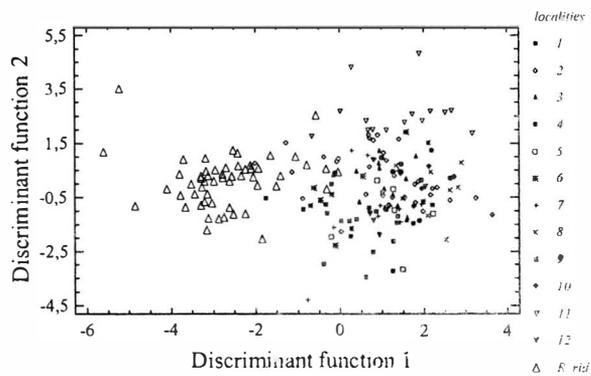


FIG. 7. Distinction among water frogs collected at 12 localities in Turkey and *Rana ridibunda* by discriminant functions. Details on the discriminant analysis are given in Table 6.

TABLE 5. Principal component analysis of the data set on 138 water frogs from Turkey and 55 *Rana ridibunda* from Kazakhstan, Armenia, and Greece consisting of eight log₁₀-transformed, standardized morphometric variables (HEADeye and FOOT were not measured in Kazakhstan and Armenia). The association (=component weights) of single variables to the derived 3 principal components representing a total of 93.4% of total variance is presented.

	PC 1	PC 2	PC 3
Eigenvalue	6.64	0.54	0.30
Variable:			
SVL	0.373	-0.069	0.011
CIL	0.345	-0.003	-0.125
DPL	0.305	0.718	-0.560
FEMUR	0.362	-0.353	-0.005
TIBIA	0.372	-0.150	-0.021
HEADmax	0.378	0.017	0.014
SNOUT-EYE	0.365	-0.240	-0.129
TYMP	0.323	0.455	0.809

increased their classification success. While all other populations showed a remarkable degree of overlap with the scores of *R. bedriagae* - indicating conspecificity - the frogs from Ulubey were certainly distinct (Fig. 5). In contrast, the frogs of Lake Beyşehir appeared to be extraordinary large *R. bedriagae*.

In a second step, we compared the external morphology of the frogs from Turkey with those of *R. ridibunda* from Kazakhstan, Armenia and Greece (Table 5). PC1 accounted for 83.0% of total variance of the pooled data set (Fig. 6A). The comparison of regression lines demonstrated that the relationship SVL/PC1 significantly differed between the frogs from Turkey and *R. ridibunda* (ANOVA: intercepts $P < 0.00001$; slopes $P > 0.05$). The shape-related PC2 and PC3 (accounting for 6.7% and 3.7% of total variance, respectively) did not resolve clear differences, but the overlap area of scores was very small between the frogs from Turkey and *R. ridibunda* (Fig. 6B). Discriminant analysis yielded five significant discriminant functions and confirmed that none of the water frog samples from Turkey was assignable to *R. ridibunda* (Table 6, Fig. 7). Remarkably, scores of the frogs from Ulubey fell outside the range of both *R. ridibunda* and the other populations from Turkey.

DISCUSSION

The morphometric data obtained along a transect including 12 localities from the Mediterranean coast to the Black Sea coast clearly confirms that a large area of Turkey is inhabited by the Levantine frog *R. bedriagae*. As expected from previous studies, the water frogs inhabiting 11 out of the 12 localities were morphometrically indistinguishable from *R. bedriagae* collected at the type locality and further sites in Syria, Jordan and Israel. Bioacoustic and morphometric studies have already provided evidence that the assignment of the water frogs of the Mediterranean coast of Turkey

to *R. ridibunda* is inappropriate, and should be changed to *R. bedriagae* (Joermann, Baran & Schneider, 1988; Schneider & Sinsch, 1992; 1999; Sinsch & Schneider, 1999). Recently, Jdeidi, Bilgin & Kence (2001) accepted this conclusion, although their multivariate study on the morphometry of water frogs from Turkey did not include reference populations of either *R. bedriagae* or *R. ridibunda* from outside Turkey. The convincing evidence from bioacoustics (Schneider & Sinsch, 1992, 1999), morphology (Sinsch & Schneider, 1999; this study) and allozymes (Beerli, Hotz & Uzzell, 1996) should finally eliminate the generalization that the water frogs in Turkey are *R. ridibunda*, and lead to a common acceptance of *R. bedriagae* as the predominating water frog species in this country.

The water frogs inhabiting the Lakes district including Lake Beyşehir (Arıkan, 1988; Atatür, Arıkan & Mermer, 1989/90; Arıkan, Özeti, Çevik & Tosunoğlu, 1994; Arıkan, Olgun, Çevik & Tok, 1998; Jdeidi, Bilgin & Kence, 2001; Budak, Tok & Ayaz, 2000) differ from the water frogs from all other sites studied in terms of maximum size (Table 7) and the striking orange colouration of the ventral skin (Schneider, 2001). These features were used by Arıkan (1988) to establish the subspecies *R. ridibunda caralitana*. Bioacoustic evidence (Schneider & Sinsch, 1999) and morphometrics (Sinsch & Schneider, 1999; this study) clearly demonstrate that species assignment has to be altered to *R. bedriagae*.

Do these frogs deserve their own taxonomic status? External coloration is very variable in water frogs, and this character alone would probably not justify subspecific status. The slight, but detectable morphometric divergence found in this study and also noticed by Jdeidi, Bilgin & Kence (2001) is basically the result of size effects in the discriminant analysis. Frogs from Lake Beyşehir with a SVL below 90 mm cannot be distinguished from *R. bedriagae*. Nevertheless, it remains unresolved whether the ability to grow to the observed large size is genetically fixed or reflects a phenotypic response to environmental conditions. The taxonomic implications of specific karyological features detected by Alpagut & Falakalı (1995) in these frogs are unclear. The haplotypes (mtDNA) of frogs collected in Alanya (typical *R. bedriagae* in external morphology) and in Beyşehir were so similar that Plötner *et al.* (2001) considered both forms as pertaining to the same taxon. In conclusion, evidence available so far may indicate an early stage of the speciation process, but it is a matter of discussion whether local differentiation has already progressed to a level which justifies an own taxonomic status. The suggestion of Jdeidi, Bilgin & Kence (2001) to assign species status is certainly not justified. However, if subspecific status is accepted, the Beyşehir frogs should be referred to as *R. bedriagae caralitana*.

The morphometric comparison of water frogs from Ulubey, in the district of Ordu (Black Sea coast), with *R. bedriagae* and *R. ridibunda*, yielded an unexpected

TABLE 6. Significant discriminant functions based on eight log₁₀-transformed morphometric variables to distinguish among water frogs from 12 localities in Turkey and *Rana ridibunda*. In (C) the values shown are actual (1st row) and predicted (2nd row) localities.

(A) Statistical significance:

Discriminant Function	Eigenvalue	Relative Percentage	Canonical Correlation	Wilks Lambda	χ ²	df	P
1	3.25	64.9	0.875	0.058	517.1	96	<<0.00001
2	0.95	18.9	0.698	0.246	254.4	77	<<0.00001
3	0.34	6.8	0.503	0.479	133.4	60	<<0.00001
4	0.18	3.6	0.392	0.642	80.4	45	0.0009
5	0.13	2.7	0.344	0.759	50.2	32	0.0216

(B) Unstandardized coefficients of the discriminant functions:

	Discriminant functions				
	1	2	3	4	5
SVL	-38.34	19.23	-3.24	-22.25	26.16
CIL	6.73	6.77	3.35	1.94	10.21
DPL	10.15	-3.89	-7.63	-11.09	-0.38
FEMUR	-16.66	-19.69	18.58	-8.69	-17.39
TIBIA	22.78	12.32	-17.07	8.69	1.25
HEADmax	5.58	1.31	0.86	38.83	-9.90
SNOUT-EYE	-0.75	-14.35	-13.94	-1.20	-12.99
TYM	10.58	9.70	15.29	-10.46	-6.46
CONSTANT	30.38	-18.83	12.66	7.03	3.02

(C) Classification success:

	1	2	3	4	5	6	7	8	9	10	11	12	<i>R. ridibunda</i>
1	14 (63.6%)	-	-	-	2 (9.1%)	2 (9.1%)	2 (9.1%)	-	-	-	-	1 (4.6%)	1 (4.6%)
2	-	6 (18.8%)	5 (15.6%)	4 (12.5%)	3 (9.4%)	2 (6.3%)	4 (12.5%)	-	4 (12.5%)	1 (3.1%)	-	-	3 (9.4%)
3	1 (7.1%)	1 (7.1%)	7 (64.3%)	1	1 (7.1%)	1 (7.1%)	1 (7.1%)	-	1 (7.1%)	-	-	-	-
4	-	1 (11.1%)	1 (11.1%)	4 (44.4%)	1 (11.1%)	-	-	1 (11.1%)	1 (11.1%)	-	-	-	-
5	1 (16.7%)	1 (16.7%)	-	-	3 (50.0%)	-	-	1 (16.7%)	-	-	-	-	-
6	-	-	-	-	-	3 (60.0%)	-	-	-	-	-	-	-
7	3 (25.0%)	2 (16.7%)	-	1 (8.3%)	-	-	4 (33.3%)	1 (8.3%)	1 (8.3%)	-	-	-	-
8	-	1 (12.5%)	-	-	1 (12.5%)	-	-	5 (62.5%)	-	1 (12.5%)	-	-	-
9	-	1 (20.0%)	-	-	1 (20.0%)	-	-	-	2 (40.0%)	-	-	1 (20.0%)	-
10	-	-	-	1 (20.0%)	-	-	-	1 (20.0%)	-	2 (40.0%)	-	1 (20.0%)	-
11	-	2 (12.5%)	-	-	-	-	-	-	-	-	14 (87.5%)	-	-
12	-	-	-	-	1 (25.0%)	-	-	1 (25.0%)	-	-	-	2 (50.0%)	-
<i>R. ridibunda</i>	1 (1.8%)	1 (1.8%)	-	-	-	3 (5.5%)	-	-	1 (1.8%)	1 (1.8%)	-	-	48 (87.3%)

TABLE 7. Distinctive morphological features of five water frog groups: *Rana bedriagae* (Turkey: localities 1, 3-9, 12), *R. bedriagae* (outside Turkey), *R. bedriagae* (caralitana, locality 2), *R. spec.* (locality 11), and *R. ridibunda* (outside Turkey). Data are given as sex-specific mean, corresponding standard error and range. P_{sex} denotes significance of difference between sexes; different letters indicate significant differences at the 0.05 level (Multiple range test with Bonferroni correction).

	<i>Rana bedriagae</i>						<i>R. spec.</i>		<i>R. ridibunda</i>	
	inside Turkey		outside Turkey		caralitana		males	females	males	females
	males n=71	females n=19	males n=41	females n=53	males n=10	females n=21				
SVL	65.2±1.3 ^a	71.6±2.2 ^a	62.3±1.3 ^a	69.1±1.9 ^a	77.4±5.0 ^b	82.4±4.4 ^b	83.9±1.0 ^b	95.0±2.7 ^c	83.5±1.6 ^b	95.2±2.9 ^c
[mm]	44.2-85.0	57.4-88.4	47.1-78.5	46.4-92.8	55.9-104.9	61.7-122.4	78.0-88.7	89.5-103.9	63.1-99.4	69.5-112.1
P_{sex}	0.0201		0.0061		>0.05		0.0003		0.0004	
Tympanum	5.7±0.1 ^b	6.2±0.2 ^b	5.3±0.1 ^a	5.7±0.1 ^a	6.5±0.3 ^b	6.8±0.3 ^b	8.4±0.4 ^c	8.2±0.4 ^c	6.0±0.1 ^b	6.4±0.2 ^b
[mm]	4.2-7.5	4.7-8.7	4.2-6.9	4.6-8.1	4.9-7.8	5.0-8.8	6.9-11.6	7.4-9.2	4.5-7.9	4.5-7.8
P_{sex}	>0.05		0.0105		>0.05		>0.05		>0.05	
Leg length/	1.54±0.01 ^a	1.48±0.02 ^a	1.54±0.01 ^a	1.52±0.01 ^a	1.51±0.01 ^a	1.52±0.02 ^a	1.49±0.02 ^a	1.39.2±0.03 ^b	1.48±0.01 ^a	1.44±0.01 ^b
SVL	1.26-1.80	1.33-1.62	1.34-1.70	1.25-1.69	1.38-1.62	1.43-1.70	1.41-1.61	1.27-1.45	1.42-1.53	1.41-1.52
P_{sex}	0.0052		>0.05		>0.05		0.0099		>0.05	
Tibia/femur	1.09±0.01 ^a	1.08±0.02 ^a	1.07±0.02 ^a	1.06±0.01 ^a	1.10±0.02 ^a	1.09±0.01 ^a	1.10±0.02 ^a	1.16±0.04 ^a	1.01±0.01 ^b	1.00±0.01 ^b
	0.80-1.30	0.93-1.21	0.91-1.26	0.88-1.26	0.96-1.16	1.01-1.15	1.02-1.16	1.04-1.26	0.85-1.13	0.90-1.09
P_{sex}	>0.05		>0.05		>0.05		>0.05		>0.05	
Callus/	0.30±0.01 ^a	0.31±0.02 ^a	0.30±0.01 ^a	0.31±0.01 ^a	0.31±0.02 ^a	0.29±0.01 ^a	0.34±0.02 ^b	0.36±0.03 ^b	0.37±0.01 ^b	0.36±0.01 ^b
1st finger	0.23-0.43	0.23-0.39	0.24-0.37	0.21-0.38	0.23-0.38	0.20-0.38	0.22-0.50	0.32-0.45	0.24-0.53	0.27-0.46
P_{sex}	>0.05		>0.05		>0.05		>0.05		>0.05	

result. As *R. ridibunda* inhabits Armenia (Schneider & Egiarjan, 1989, 1991; Schneider & Sinsch, 1992; Sinsch & Schneider, 1999), it seemed reasonable to assume that the geographical range of these frogs also extends to Turkey, specifically to the Black Sea coast and thus to Ulubey. The geographical distance between Ulubey and Hankavan – the study site near Lake Sevan in Armenia – is about 570 km. Nevertheless, the multivariate analyses of morphometric data leaves no doubt that the water frogs collected at Ulubey are neither conspecific with *R. ridibunda* or *R. bedriagae*. They are easily distinguished from *R. bedriagae* and *R. ridibunda* by a larger tympanum diameter (Fig. 4, Table 7). There is also non-morphological evidence that water frogs from Ulubey are different from those of other regions in Turkey. In his comparative study on water frogs from Lake Beyşehir, Lake İznik (north-west Turkey), Malatya (eastern Anatolia) and Ulubey, Arıkan (1990) analysed features of external morphology and of blood cells. The Ulubey frogs differed from the other three samples studied (*R. bedriagae*) in the number of erythrocytes and leucocytes as well as with respect to DNA-content.

Taken alone, these observations seem to indicate that the water frogs from Ulubey represent a new taxon. However, considering that these frogs were collected in 1984 (by Yılmaz), one of us (H. Schneider) visited the village of Ulubey in May, 2001. No water frog was heard calling or captured during this visit. Thus, the

present status of the water frog population near Ulubey is unclear; it may have gone extinct. Turna Suyu – about 30 km distant from Ulubey – was the nearest site at which water frogs were found during our visit. To our surprise, neither these frogs nor those from Kızılırmak at Baflra – about 220 km distant from Ulubey – morphometrically resembled those of the Ulubey sample, but were clearly conspecific with *R. bedriagae*. There are at least two alternative hypotheses to explain these contradictory observations: (1) the water frog population from Ulubey consisted of ordinary *R. bedriagae* which were altered in response to an unknown environmental impact (e.g. pesticides); (2) the Ulubey frogs represent a new taxon, more closely related to *R. bedriagae* than to *R. ridibunda*. If the second hypothesis is true, we would expect to find other frog populations in the region of Ordu which share the characteristics of the Ulubey frogs.

The most surprising result of our morphological survey is the fact that we were unable to establish the presence of *R. ridibunda* in Turkey. The same was true for a preliminary mtDNA-analysis of 10 frogs collected at six sites in Turkey (Plötner *et al.*, 2001). The external morphology of all frogs strongly deviated from that of western *R. ridibunda* in Thrace (Greece) and eastern *R. ridibunda* in Armenia. However, a parallel bioacoustic survey (Schneider & Sinsch) which will be published in a separate paper, yielded different results. At several central and northern localities frogs morphometrically

assigned to *R. bedriagae* gave advertisement calls assignable to *R. ridibunda*, whereas at southern and western localities morphometric and bioacoustic assignment coincided. Thus, the analysis of more than one character complex seems to be necessary to fully appreciate the biogeography of the water frogs in Turkey and to propose a reliable taxonomic reassessment.

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APPENDIX 1

Geographical origin of specimens examined morphometrically. The numbers of the localities in Turkey refer to Fig. 1; details on the geographical locations of the reference populations of *R. bedriagae* and *R. ridibunda* are given in Sinsch & Schneider (1999). Institutional abbreviations are as follows: SMF, Senckenberg Museum Frankfurt; ZDEU, Zoology Department, Ege University; ZFMK, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn.

TURKEY

1. *Alanya* (Antalya). Sample 1: 5 males, 5 females, ZFMK 40192-40201, collected in April 1983. Sample 2: 1 male, 1 female, unpreserved, collected by H. Schneider in April 1994. Sample 3: 10 males, unpreserved, collected by H. Schneider in April 1996.
 2. *Lake Beyşehir* (Konya, type locality of *R. ridibunda caralitana* Arıkan, 1988). Sample 1: 2 males, 10 females, ZDEU 2/982-1 to -12, collected by H. Arıkan in April 1981. Sample 2: 4 males, 4 females, ZDEU 8/982-1 to -8, collected by H. Arıkan in April 1982. Sample 3: 1 male, 1 female, unpreserved, collected by H. Schneider in April 1994. Sample 4: 2 males, 8 females, unpreserved, collected by H. Schneider in April 1996.
 3. *Yakaköy*, 7 km northwest of Dinar (Afyon). Sample 1: 5 males, unpreserved, collected by H. Schneider in June 1998. Sample 2: 9 males, unpreserved, collected by H. Schneider in May 1999.
 4. *Beydilli*, Lake Işıklı (Denizli). 2 males, 7 females, unpreserved, collected by H. Schneider in May 1999.
 5. *Çayırpınar*, Lake Eber (Afyon). 6 males, unpreserved, collected by H. Schneider in June 1998.
 6. *Çifteler* (Eskişehir). 4 males, 1 female, unpreserved, collected by H. Schneider in June 1998.
 7. *Balçıkhisar*, ca. 30 km north of Çifteler (Eskişehir). 9 males, 3 females, unpreserved, collected by H. Schneider in May 1999.
 8. *Çakırca*, Lake İznik (Bursa). Sample 1: 1 male, unpreserved, collected by H. Schneider in June 1998. Sample 2: 7 males, unpreserved, collected by H. Schneider in May 1999.
 9. *Karasu* (Adapazarı). 5 males, unpreserved, collected by H. Schneider in June 1998.
 10. *Kızılırmak* at Bafra (Samsun). 3 males, 1 female, 1 juvenile, unpreserved, collected by H. Schneider in May 2001.
 11. *Ulubey* (Ordu). Small pond at the entrance to the village. 11 males, 5 females, ZDEU 2/984-1 to 16, collected by İ. Yılmaz in April 1984.
 12. *Turna Suyu* (Ordu). 4 males, unpreserved, collected by H. Schneider in May 2001.
1. REFERENCE POPULATIONS OF *RANA BEDRIAGAE*:
- Syria*:
- Jebel el Ansariye*. 1 male, 3 females, ZFMK 60901-6904.
- Ar Raqqah*. 1 male, 3 females, SMF 75349-75352, collected by H. Martens
- Nahr al-Habur*. 4 males, 2 females, SMF 73715-73717, 73721, 73723-73724, collected by H. Martens in October 1988.
- Abu Kemal, Euphrates River*. 4 males, ZFMK 61785-61788.
- Bahrat Khatuniyah*. 1 male, 3 females, SMF 75467-75470, collected by H. Martens.
- Barada River, surroundings of Damascus* (type locality of *R. bedriagae*). 1st sample: 1 male, SMF 5900, collected by H. Simon 1882; 2nd sample: 4 males, 2 females, SMF 75688-75689, 75693-75696, 75699, collected by H. Martens.
- Quanwat, Jebel Al-Arab*. 2 males, 3 females, SMF 75610-75613, collected by H. Martens
- Mzeirib*. 4 males, 8 females, SMF 75644-75655, collected by H. Martens.
- Jordan*:
- Zarqa*. 4 males, 2 females, SMF 76454-76459.
- Wadi Wala*. 6 females, SMF 76469-76474.
- Israel*:
- Birket Ata, Hadera*. 4 males, 5 females, ZFMK 52836-52844, collected by E. Nevo in 1992.
- Jericho*. 11 males, 17 females, ZFMK 52836-52844 and unpreserved, collected by E. Nevo in 1992.
2. REFERENCE POPULATIONS OF *RANA RIDIBUNDA*:
- Kazakhstan*:
- Atyrau*, formerly Guryev (type locality of *R. ridibunda*). 2 males, 2 females, unpreserved, collected by H. Schneider and E. M. Egiasarjan in May 1990.
- Armenia*:
- Hankavan*. 19 males, 11 females, unpreserved, collected by H. Schneider and E. M. Egiasarjan in May 1990.
- Greece*:
- Ardas, Thrace*. 2 males, 1 female, unpreserved, collected by T. S. Sofianidou in March 1990.
- Valtos, Thrace*. 12 males, 6 females, unpreserved, collected by T. S. Sofianidou in March 1990.

THE EFFECTS OF COHORT STRUCTURE AND DENSITY ON LARVAL GROWTH AND DEVELOPMENT IN *ALYTES MULETENSIS* : IMPLICATIONS FOR CONSERVATION

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The Mallorcan midwife toad (*Alytes muletensis*) has a very extended breeding season, and the nature of competition between larvae in the torrent pools where it breeds is likely to change across time. Larvae commonly overwinter and grow to a very large size and new hatchlings will have to compete with these overwintered larvae under varying conditions of density. The effects of density and cohort structure (i.e. the presence/absence of large overwintered tadpoles) on the growth and development of hatchling *A. muletensis* larvae were investigated in the laboratory using a factorial design. Large competitors, high densities and lower temperatures were all shown to suppress tadpole growth and development. Larger competitors were superior, especially the very large overwintered tadpoles. Whilst large size is advantageous, avoiding competition with overwintered tadpoles or high densities of tadpoles is probably much more important in determining size at – and timing of – metamorphosis. Because *A. muletensis* is an endangered species, knowledge of life history constraints can guide management of wild populations. The results are discussed in terms of potential optimal times to breed in light of the changing competitive environment.

Key words: amphibian, anuran, competition, density effects, tadpoles

INTRODUCTION

The Mallorcan midwife toad (*Alytes muletensis*) is an endangered species (Groombridge, 1994) and knowledge of life-history constraints can guide the management of wild populations. To date, however, there is little information on a fundamentally important part of its life cycle, namely the larval environment (but see Schley, 1996; Griffiths *et al.*, 1998). In many anurans, the effects of competition in the larval environment can carry through to the juvenile and adult stages (Berven, 1990). A large tadpole will become a large metamorph which may have better physiological performance (John-Alder & Morin, 1990), become a larger adult or, together with early metamorphs, reproduce sooner, and may ultimately survive longer (Berven & Gill, 1983; Smith, 1987). Conversely, late metamorphosis will leave less time for terrestrial feeding and juveniles may not be able to accumulate enough energy to see them through the winter (Berven & Gill, 1983) unless they can compensate for poor aquatic growth by feeding rapidly on land (i.e. 'catch up' growth in species with extended juvenile periods, Halliday & Verrell, 1988). Larval competition can thus be the most important determinant of fitness at all stages of the life cycle.

A. muletensis larvae do not necessarily metamorphose at the end of a breeding season but sometimes overwinter as tadpoles which, by the beginning of the following season, have reached a very large size (65-88

mm long, Bush, 1993 and Lea, pers. obs). Therefore, new hatchlings (11-15 mm long) may have to compete for resources with these much larger tadpoles. In *Bufo woodhousii*, another prolonged breeder, tadpoles hatching late in the season must compete with larger conspecifics, and this suppresses the developmental rate and lowers the chances of survival of the smaller larvae (Woodward, 1987). The enormous size range found in *A. muletensis* and the very extended breeding season (5-7 months) may mean that the effects of competition with larger larvae could be considerable.

A high density of tadpoles can also affect fitness; usually prolonging development and increasing the variation and reducing the average size at metamorphosis, as well as reducing larval survival (Richards, 1958; Brockelman, 1969; Wilbur, 1977; Buskirk & Smith, 1991). Conversely, some authors have shown that an increase in density can be beneficial, actually enhancing growth (Beiswenger, 1975; Breden & Kelly, 1982) via 'facilitation', whereby many interacting tadpoles stir up more food from the benthos and individuals exhibit higher rates of feeding behaviour in larger groups.

Previous experiments with *Alytes* have produced contradictory results with respect to the effects of density on larval growth. There is some evidence that *Alytes* tadpoles grow better in crowded conditions than under conditions of lower density. *Alytes obstetricans* larvae reared in isolation grow and develop slower, show more variation in the timing of metamorphosis, and metamorphose at a lower size than tadpoles reared in small groups, thus indicating some facilitation in this species (Guyotant, 1970). To date there have been no papers

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published on density effects in *A. muletensis*. This paper explores the potential consequences of competition in the larval environment for *A. muletensis*, looking at the effects of the size of competitors and the number of competitors on larval growth and development.

MATERIALS AND METHODS

Tadpoles were bred in captivity from wild Mallorcan adults that formed part of a captive breeding programme. They were measured and staged weekly for 98 days after hatching. There were two density conditions: 'high' (H) (eight larvae per container) and 'low' (L) (two larvae per container). There were also two cohort conditions: 'all small' (s) (all the larvae were small i.e. all hatched on the day that the treatments were started) and 'half larger' (l) (half the larvae were larger i.e. half were one-year-old tadpoles, over-wintered from the previous season). Thus, there were four treatments in total: 'Hs' (high density – all small); 'Hl' (high density – half larger); 'Ls' (low density – all small); and 'Ll' (low density – half larger; Fig. 1).

There were four replicates of each treatment (a total of sixteen replicates), which was the maximum number practicable due to constraints on laboratory space. The limited availability of hatchlings at any one time (because of the protracted breeding and small clutch size of this species) (clutch size = 11 ± 3 eggs, Lea pers. obs.) meant that replicates 1 and 2 had to be started earlier (16 June 1997) than replicates 3 and 4 (started on 23 July 1997). The decreasing temperature later in the summer (water temperature at noon ranged from 22.1 to 16.4 °C over the course of the experiment) led to slower growth and development of the tadpoles in replicates 3 and 4 compared to replicates 1 and 2; this is referred to as the effect of 'season' in the analysis.

Tadpole length was measured using a V-shaped trough made from two rulers glued together at 45° and sealed (with a zero mm mark) at each end. Each tadpole was placed in the trough with just enough water to cover it and a mouth to tip of tail measurement was taken when its mouth touched either end of the trough. Measurements were taken blindly to a precision level of 0.5 mm and error was estimated at ± 0.1 mm. Length (logged) was a good predictor of overall size as shown by a strong correlation with (logged) volume of water displaced (Pearson's $r=0.99$, $df=18$, $P<0.001$).

Development was mapped using the stages described for *A. obstetricans* (Cambar & Martin, 1959). The patterns of differentiation for metamorphosing *A.*

muletensis and *A. obstetricans* larvae are exactly the same; the only differences are in rate and stage-specific size. The stages relevant to the experiment as categorised by Cambar & Martin (1959) were 'IV5' through to 'IV15'. IV5 is the free-living tadpole upon hatching from the egg; IV15 describes the point at which the buccal denticles start to diminish and aquatic feeding behaviour consequently begins to alter. IV15 is easily determined, because at this point the hind limb bud elongates and gets its characteristic bending, and tadpoles have stopped increasing in length but have not yet started to reabsorb their tails. Thus IV15 was deemed an appropriate point to stop taking measurements.

The extended development in this species, together with the late start of the experiment relative to the onset of the breeding season, meant that many of the tadpoles had not completed metamorphosis by the end of the experiment. Because of this, a reference point – day 42 after hatching – was used to compare the size and developmental stage of the tadpoles under the different regimes. Day 42 was used because this marked the point where the fastest developing tadpole in the experiment reached stage IV15.

For each set of replicates hatchling tadpoles were drawn from a minimum of three clutches, each from a different female, in order to minimise any differences in genetic or maternal (egg quality) effects on growth and differentiation. Tadpoles were randomly selected for each treatment by collecting those that swam freely into a net fixed in the centre of a holding tank. Any particularly large or small hatchlings were discarded to reduce the variation in tadpole size at the start of the experiment. Tadpoles were used on the day they hatched. Any overwintered tadpoles that started to undergo metamorphosis were removed and replaced with similar sized competitors.

Tadpoles were kept in plastic aquaria (12.5 x 26.5 x 16 cm) filled to 1 cm below the rim with 3 litres of aged tap water. These were placed on a lab bench, at room temperature and exposed to a natural photoperiod, and their relative positions were altered randomly twice weekly, while water changes and feeding were carried out weekly. Water temperature, pH and dissolved oxygen levels were monitored (using: Piccolo ATC pH meter, Hanna Instruments, BDH DigiThermo temperature meter and Aquamerk oxygen kit # 11107) and none of the variables were found to differ between the treatments (temperature: $F_{3,36}=0.36$; pH: $F_{3,36}=0.74$; oxygen: $F_{3,36}=0.67$; $P>0.05$ in all cases).

Faeces were collected regularly from all the aquaria, squashed and diluted in a drop of distilled water, and examined under the microscope for the presence of *Anurofeca* (previously *Prototheca*; Beebee & Wong, 1992; Beebee, 1995). Food ration (35 mg of crumbled 'Tetra Min' fish food per tadpole per week) was calculated from trials measuring the maximum food clearance rate of the larger tadpoles, hence there was *ad libitum* food at all times. Tadpole density was determined in the high-density replicates by the maximum amount of food

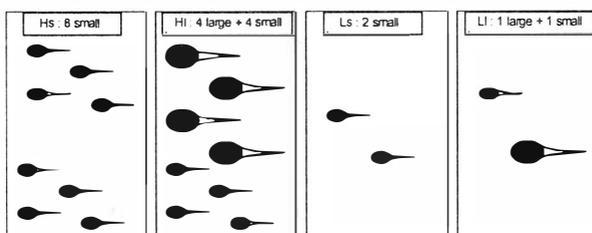


FIG. 1. Cohort and density treatments - see text.

that could be applied to each aquarium before visible fouling of the water occurred. This quantity was then divided by the per capita ration and rounded down to give an estimate of what would be a suitable maximum density. Two tadpoles were used in the low density treatments to provide an adequate contrast, while limiting any possible negative effects on growth that may occur by housing tadpoles singly (e.g. a lack of facilitation).

STATISTICAL ANALYSIS

Only the 'small' tadpoles were measured as these were the 'targets' of competition. There were three variables used in the analyses:

Variable 1. Individual tadpole sizes at day 42.

Variable 2. Increment in size at day 42, calculated as: (individual tadpole size at day 42 - mean tadpole size in replicate at day 0) / mean tadpole size in replicate at day 0.

Variable 3. Mean developmental stage reached by day 42 in replicates 1 and 2 only (see below).

Three-way ANCOVA or ANOVA were used to determine whether the treatment effects (density, cohort and season) differed significantly for variables 1 and 2 respectively. The two analyses (ANCOVA on variable 1, ANOVA on variable 2) both measure the treatment effect on size at day 42 whilst controlling for size at day 0. Agreement between them therefore lends support to any conclusions.

Two-way ANCOVA was used to determine whether the effects of density and cohort differed significantly for variable 3. Although stage data are ordinal, according to the 'central limit theorem' means should be normally distributed even if the original data are not (Sokal & Rohlf, 1980). Only replicates 1 and 2 were analysed because most of the tadpoles in replicates 3 and 4 were at the same developmental stage at the end of the experiment. (By omitting replicates 3 and 4 from the analysis the data conformed to the assumptions of the ANCOVA).

The mean size of tadpoles in each replicate at day 0 was used as a covariate in the ANCOVAs, and also in the equation used to calculate variable 2, because individual tadpoles could not be identified during the experiment.

RESULTS

SIZE AT DAY 42

Single effects. Growth rate slowed dramatically as the summer progressed (Fig. 2, Table 1), and tadpoles in the later replicates (3 and 4) were much smaller than those in the earlier replicates (1 and 2) (effect of season: $F_{1,51}=38.9$, $P<0.001$). Seasonal effects apart, the presence of larger competitors had the most significant effect on tadpole size at day 42. Larvae were much larger in the 's' treatments (all small tadpoles) than they were in the 'l' treatments (with larger competitors) (effect of cohort: $F_{1,51}=8.78$, $P<0.01$). Fig. 2 also shows

that there was far more variation in growth in the 'l' treatments than in the 's' treatments. (For high density treatments, the CV of 'l' treatments = 19.1%, whilst the CV of 's' treatments = 11.4%; mean sizes are 31.2 mm and 37.3 mm respectively). The next most significant single effect was density. At day 42, tadpoles were larger in the low-density treatments ('L') than they were in the high density treatments ('H') (effect of density: $F_{1,51}=7.53$, $P<0.01$). Starting size also had a significant effect on size at day 42, although this was less important than either density or cohort (effect of mean size at day 0: $F_{1,51}=4.42$, $P<0.05$).

Combined effects. Density had an effect regardless of season (there was no significant interaction between density and season: $F_{1,51}=0.91$, $P>0.05$). Tadpoles showed the same trend for both the early (1 and 2) and

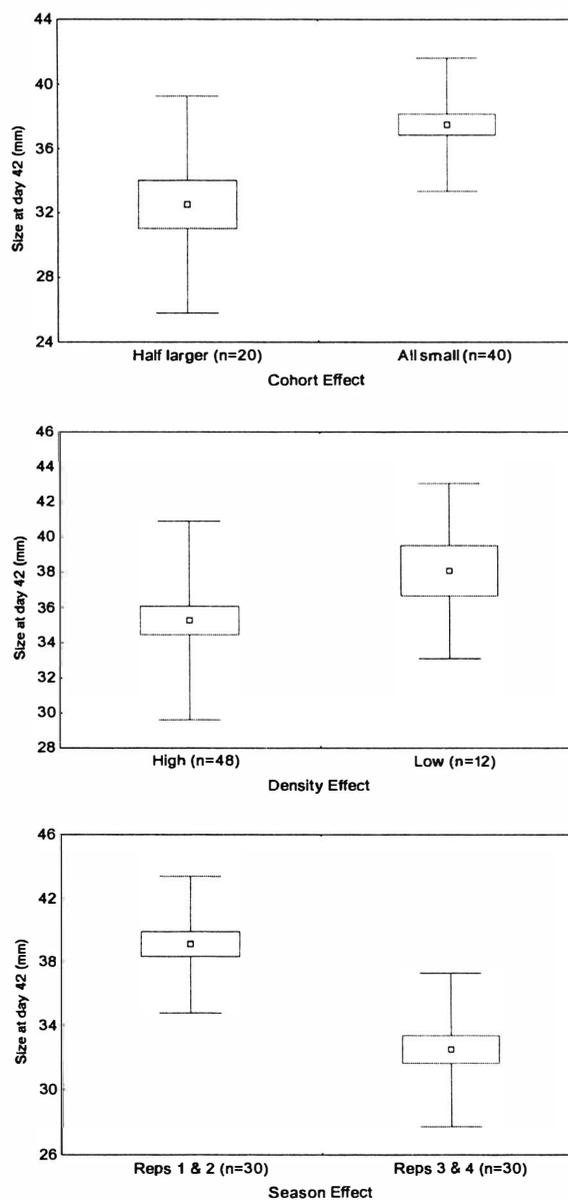


FIG. 2. Effects of cohort (top), density (middle) and season (bottom) on size at day 42. Whiskers are \pm SD, boxes are \pm SE, small square is mean, y-axis units are mm.

TABLE 1. Results of ANOVA or ANCOVA on size, stage and increment at day 42. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS $P > 0.05$.

	SIZE		STAGE		INCREMENT	
	df	F	df	F	df	F
COVARIATE						
Mean size at day 0	1	4.4 *	1	1.9 NS	-	-
EFFECTS						
Cohort	1	8.8 **	1	11.2 *	1	9.3 **
Density	1	7.5 **	1	2.0 NS	1	2.2 NS
Season	1	38.9 ***	-	-	1	21.8 ***
INTERACTIONS						
Cohort x Density	1	4.7 *	1	2.2 NS	1	1.4 NS
Cohort x Season	1	5.9 *	-	-	1	2.1 NS
Density x Season	1	0.9 NS	-	-	1	1.6 NS
Cohort x Density x Season	1	1.4 NS	-	-	1	0.3 NS
RESIDUAL	51	-	3	-	52	-
TOTAL	59	-	7	-	59	-

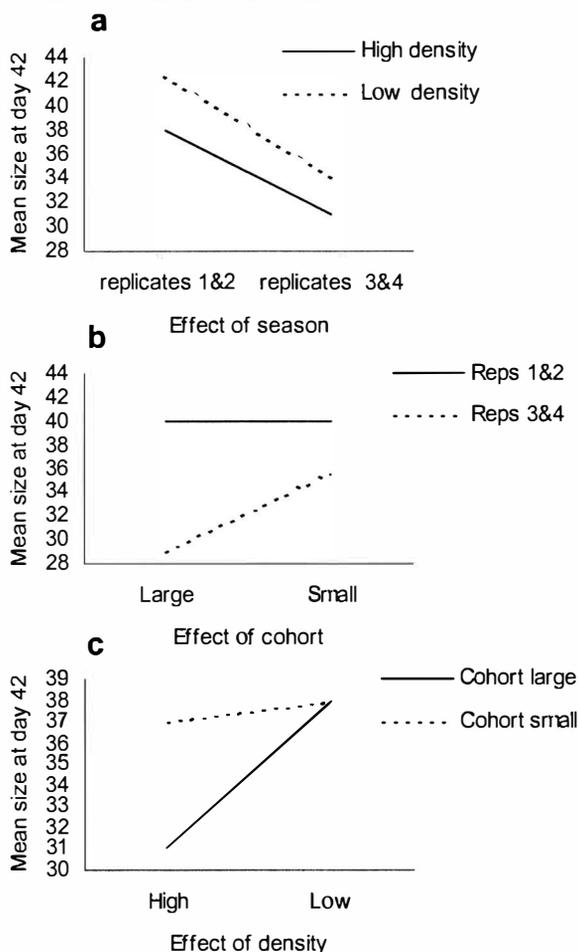


FIG. 3. Interactions (for size, in mm, at day 42) between: a, density and season (density had an effect that was independent of season i.e. in both the early and late replicates tadpoles were larger under low density conditions); b, cohort and season (the effects of cohort and season interacted, such that the presence of large competitors had a greater growth suppressing effect in the later replicates); c, density and cohort (the effects of cohort and density interacted, such that the presence of large competitors had a greater growth suppressing effect when combined with high density).

late (3 and 4) replicates in that they were larger under low densities than they were under high densities (Fig. 3a). There was an interaction between cohort and season ($F_{1,51} = 5.86$, $P < 0.05$). Cohort had a large effect in replicates 3 and 4 (with the 'large' cohort suppressing growth) but had no effect in replicates 1 and 2 (Fig. 3b). There was also an interaction between cohort and density ($F_{1,51} = 4.72$, $P < 0.05$). The growth suppressing effect seen in replicates with large competitors ('cohort large', Fig. 3c) was much greater at high than at low density. High density had an independent effect on reducing mean size at day 42 without large competitors being present ('cohort small'). Also, there was no difference between the replicates with or without large competitors at low density (probably due to the small sample size of the low-density replicates), but there was a compounding effect on growth suppression when large competitors were combined with high density.

No *Anurofeca* were found in the present study. However, they have been found in the faeces of captive-reared *Alytes muletensis* tadpoles before (R.Griffiths, pers. com.).

STAGE AT DAY 42

None of the tadpoles in replicates 3 or 4 had started to approach metamorphic climax by day 42, and all except for one individual were still at stage IV6 (that individual had only reached stage IV7, Fig. 4). Replicates 3 and 4 are not included in the following analysis as they introduce skew into the data set.

The only significant effect on mean stage was that of cohort ($F_{1,3} = 11.16$, $P < 0.05$). The mean stage in the treatments without larger competitors was 9.3 ± 1.09 , which compares to 6.3 ± 0.24 when larger competitors were present. The effect of the covariate 'mean size at day 0' was not significant ($F_{1,3} = 1.9$, $P > 0.05$). The effect of density was not significant (mean stage: high-density treatments = 7.1 ± 0.99 , low-density treatments =

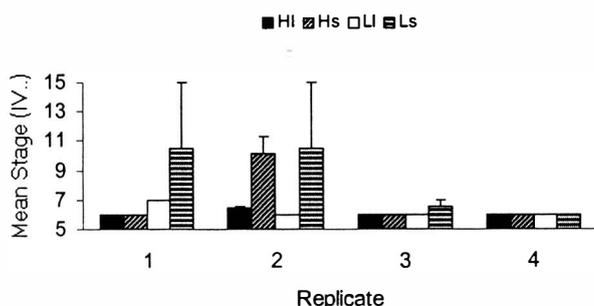


FIG. 4. Mean stage at day 42. Stages are IV5 to IV15 from Cambar & Martin (1959). Error bars show standard errors.

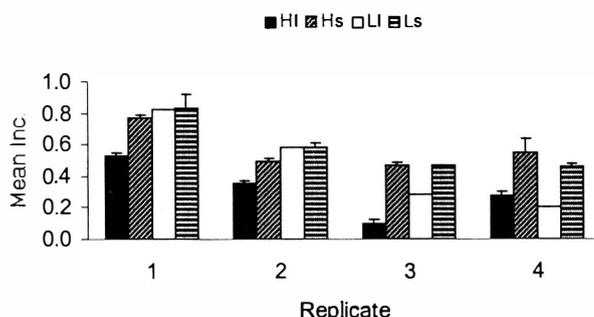


FIG. 5. Mean size increment at day 42. Units for mean increments are mm; error bars show standard errors.

8.5 ± 1.17 , $F_{1,3} = 2.0$, $P > 0.05$). This lack of significant difference between the high and low-density treatments is influenced by the fact that some tadpoles in Hs2 had reached stage IV15 by day 42 (Fig. 4). It is noteworthy that tadpoles in Hs2 had started off at a relatively large size (mean size at day 0 = 27.6 mm) but that tadpoles in other Hs treatments, which were matched in starting size with corresponding Ls treatments, took considerably longer to reach any stage than those in the Ls treatments. The interactions were not significant (Table 1).

INCREMENT IN SIZE AT DAY 42

As with size at day 42, the data on size increment show a marked difference between sets of replicates, with the early replicates showing a far greater increment in size than the later replicates (effect of season: $F_{1,52} = 21.8$, $P < 0.001$, Fig. 5). Growth increment was also suppressed by the presence of larger competitors with cohort once again having a highly significant effect (effect of cohort: $F_{1,52} = 9.3$, $P < 0.001$). Density, however, had a non-significant effect on size increment ($F_{1,52} = 2.2$, $P > 0.05$). There were no significant interactions (Table 1).

DISCUSSION

Competition clearly has adverse effects on *A. muletensis* larvae. Tadpoles suffer reduced growth and development, and the variation in their sizes increases, when they have to compete with large overwintered tadpoles. This may have arisen because the larger tadpoles monopolised most of the food and the hatchlings varied in their ability to compete for the remaining food. This, however, seems unlikely because food level was de-

signed to be *ad libitum*. Therefore, the release of growth inhibitors (Richards, 1958; 1962; Beebee & Wong, 1992; Beebee, 1995; Griffiths, 1995; Petranks, 1995) or other interference competition (Akin, 1966; Steinwascher, 1979; Alford, 1998; Faragher & Jaeger, 1998), by the large larvae over the small larvae, cannot be ruled out.

In replicates 3 and 4 large tadpoles inhibited the growth of smaller tadpoles, but in replicates 1 and 2 there was little or no effect (Fig 5). This probably arises because the higher temperature in replicates 1 and 2 stimulated the tadpoles to start metamorphosing and, because these tadpoles were differentiating at a fast rate, little energy was being diverted to somatic growth. In replicates 3 and 4, however, tadpoles were not differentiating, and all available energy was being utilised for growth. Thus the relative effects of growth suppression were much higher in replicates 3 and 4 than in replicates 1 and 2. These results are consistent with models that state that growth and differentiation rates are independent, that resources are allocated to growth after they are allocated to differentiation, and that differentiation rate is fixed early in development (Smith-Gill & Berven, 1979; Leips & Travis, 1994).

The combination of large cohort and high density drastically retarded both growth and development in all the replicates. High density also had an important independent effect on reducing final size, but the non-significant effect on size increment and stage was probably due to the small sample size and the fact that the food ration was calculated on the clearance rate of the larger tadpoles. This meant that, even in the most competitive situations, there was sufficient food to allow some growth of even the smallest tadpoles. It is likely that density would have had a greater effect under conditions of higher food stress. Data on wild densities are few but two estimates are 20 and 59 tadpoles per m^2 (R. Griffiths pers. com.). However, nothing is known on food abundance in wild pools.

The influence of starting size was significant in determining final size but it had less of an effect than cohort, density or season. From this it seems likely that any advantages of being a large hatchling would only manifest themselves under conditions of low competitive stress. Thus, while hatchling size is obviously important, the timing of tadpole deposition into the pools is probably more so. Being a large hatchling is advantageous, but not having to compete with overwintered tadpoles or high densities of tadpoles is more important in determining size and timing of metamorphosis.

The timing of breeding for prolonged breeders, especially with overwintering larvae, is very important because the nature of the competitive environment will change over time (Collins, 1979). *A. muletensis* breed from March-April to September a peak of activity in July-August (Bush, 1993), and metamorphic climax can take from a few days to two weeks depending on temperature (Lea, laboratory pers. obs). Males brood the eggs and carry them to water when the tadpoles are

ready to hatch. The low temperatures early in the season will lengthen the brooding period (Bush, 1993) and eggs will only gradually be deposited into the pools. Tadpole density should be low at this time but larvae may have to compete with the (large) overwintered tadpoles from the previous season. Competitive release (Travis, 1984) will occur as overwintered tadpoles start to metamorphose in mid-late May and, although density is increasing, hatchlings at this time should develop at a faster rate than earlier ones because of increasing temperature. By mid-June or July breeding is rapid and tadpole density will be high, there will also be some large overwintered tadpoles left that have not yet metamorphosed (Bush, 1993; Schley, 1996); competition at this time should be at its most intense. Late July-early August sees the rapid metamorphosis of large numbers of tadpoles, and whilst tadpoles are still being deposited, the overall density probably starts to decline. This again is a point of competitive release, and hatchlings should fare a little better. By September water temperature has dropped below 18°C (Bush, 1993 - no data on variation between pools and years), and below this temperature in the laboratory metamorphosis ceased (replicates 3 and 4), so tadpoles are perhaps now forced to overwinter.

In the field, a reduction in size, or lengthened larval period, may well reduce larval survival because of the increasing cumulative risks of predation (e.g. Heyer, McDiarmid & Weigman, 1975; Brodie & Formanowicz, 1983; Travis, Keen & Julianna, 1985), or being 'washed out' of the pools (Schley, 1996). Consequently, avoiding high densities and overwintering tadpoles should be advantageous. Also, breeding late in the season and prolonging the larval period may reduce lifetime reproductive success because reaching sexual maturity is dependent on a minimum age after metamorphosis in *A. muletensis* (Bush, 1993), and overwintered tadpoles will therefore breed one year later than hatchlings that do not overwinter. However, overwintered larvae would become large metamorphs and so larger, more fecund and perhaps more viable adults. Whether there is a size advantage to overwintering depends upon the relative growth rates in the aquatic and terrestrial environments (Werner, 1986). Similar trade-offs to the timing of breeding exist in *Salamandra salamandra*, where early or late cohorts are more successful depending on the amount of rainfall (Warburg, 1992; Griffiths, 1997).

The arguments above suggest that there should be selection to favour breeding during periods of competitive release (either side of mid-season) and perhaps avoid breeding at the end of the season, when hatchlings must overwinter in water and miss out on one year's potential reproduction. However, the iteroparous nature of the females (within a season) and the limited availability of sexually receptive males (Bush 1993) may prolong the reproductive period, such that toads breed at optimal and sub-optimal times. Also, asynchronous breeding, and variation in competitive ability, means that tadpoles metamorphose over an extended period, which reduces the risk that a catastrophic event (e.g. flooding) will decimate the entire population (Griffiths, 1997).

Aquatic competition could affect population structure in the field by suppressing the growth of some larvae who would then possibly be smaller at first reproduction, and thus produce smaller hatchlings (Bush, 1993), which in turn may fare poorly under conditions of high competition or predation in the pools. Intense larval competition could also reduce larval or juvenile survival, and hence adult recruitment. Bearing in mind that *A. muletensis* is endangered; knowledge of the nature of larval competition can aid management strategies. For instance, the best times to introduce translocated tadpoles into new pools may be either side of mid-season during the periods of competitive release. Further field work needs to determine the relative predation pressures in the aquatic and terrestrial environments; how predator density and food availability changes across the season; and the relative contributions of larval and juvenile growth to adult size. Only with this information can we hope to determine what the optimal strategies are, for individuals seeking to maximise their fitness.

ETHICAL NOTE

Tadpoles used in the experiments came from a captive breeding and release programme that forms a component of the recovery programme for *A. muletensis*. No animal was deliberately harmed or killed for the purposes of this experiment.

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INVASIVE POPULATIONS OF *XENOPUS LAEVIS* (DAUDIN) IN CHILE

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Invasive populations of *Xenopus laevis* are known from the UK, USA and Chile, although there is poor documentation of the latter. Currently, four administrative Regions in Chile are reported as having established populations. Fieldwork during the austral winter of 2001 was conducted in order to assess the density and diet of *X. laevis* populations in two localities. At one site, fewer than 30 adults were captured and a population 4 times this size was estimated. At the second site, nearly 2000 adults were trapped and a population of nearly 20 000 was estimated. This yielded density estimates of 0.37 and 0.25 clawed frogs m⁻² respectively. However, significant bias in the sex ratio of animals caught at each site suggests that the populations may be even larger. Stomach contents of a sub-sample of animals revealed a diet consisting primarily of zoobenthic and zooplanktonic components. Further work is required to assess the extent to which this anuran affects the biodiversity of indigenous aquatic invertebrate, fish and amphibian populations.

Key words: African clawed frogs, exotic species, invasive amphibians, South America

INTRODUCTION

The African clawed frog, *Xenopus laevis* (Daudin), is regarded as a standard for amphibian studies; renowned for its ease of laboratory maintenance and simple stimulation of breeding (Gurdon, 1996). *X. laevis* has a wide natural range, covering most of sub-Saharan Africa with five commonly recognized subspecies: the largest of these, *X. l. laevis*, is commonly encountered in South Africa, Swaziland, Lesotho, Zimbabwe, Botswana and Namibia (Poynton, 1964; Kobel *et al.*, 1996). Within its native range, this subspecies is noted for its rapid invasion of man-made water bodies and anthropogenically disturbed areas (Evans *et al.*, 1997), and in the Cape region it has displaced the naturally occurring acid black-water species *X. gilli*, contributing to the inclusion of the latter in the red data book (Picker & DeVillers, 1989).

There have been a number of publications concerning extralimital populations of *Xenopus laevis* (all of which are of *X. l. laevis* and will be hereafter referred to as *X. laevis*), and these were reviewed by Tinsley & McCoid (1996). Since then, a number of studies have raised concern that the size and impact of *X. laevis* populations are increasing in the UK and USA (Measey, 1998, 2001; Crayon, in press). Indeed, concerns over predation pressure on native aquatic invertebrates and vertebrates by introduced populations of *X. laevis* seem to be well founded (Lafferty & Page, 1997).

In this study, we begin by reviewing the literature concerning introduced populations of *X. laevis* in Chile, to determine the areas affected by invasion. Literature on other invasive amphibians concurs that impact is

positively related to density in extralimital habitats (e.g. Kupferberg, 1997; Kraus *et al.*, 1999; Crossland, 2000). Therefore, we have concentrated on population density of *X. laevis*, using two sites in the Metropolitan Region of Chile: Antumapu (studied by Lobos *et al.*, 1999) and Rinconada (a previously undocumented locality for *X. laevis*). Diet analyses were carried out on a sub-sample of animals captured.

LITERATURE REVIEW OF *XENOPUS LAEVIS* IN CHILE

Chile is a recognized biodiversity hotspot (Myers *et al.*, 2000), and has 43 known species of anurans, of which 33 are endemic to this narrow strip on the west coast of South America (Formas, 1995). The clawed frog was first mentioned in the Red Data book for Chile by Glade (1983), who called for studies to investigate the effects of this invading anuran. Veloso & Navarro (1988) also mentioned *X. laevis* in their summary of reptiles and amphibians of Chile, giving only general geographic and ecological distributions: the administrative region 'Metropolitan' (Santiago and surrounds), and semi-arid Mediterranean regions (altitude 250-500 m). Jaksic (1998) and Hermosilla (1994) described *X. laevis* from the Metropolitan and VIIIth Regions (Fig. 1), dating the entry of this species into Chile to the 1970s.

National notification of the first feral populations from Pudahuel, Metropolitan Region (Fig. 1) was made in 1986, and hope was expressed that animals would not move south (Hermosilla, 1994). However, it is thought that *X. laevis* were sent to the Department of Biology, University of Concepción (VIIIth Region) for experimentation and breeding, and subsequently escaped (Hermosilla, 1994). Formas (1995) and Hermosilla (1994) both raised concern about the possible impact of *X. laevis* on native herpetofauna.

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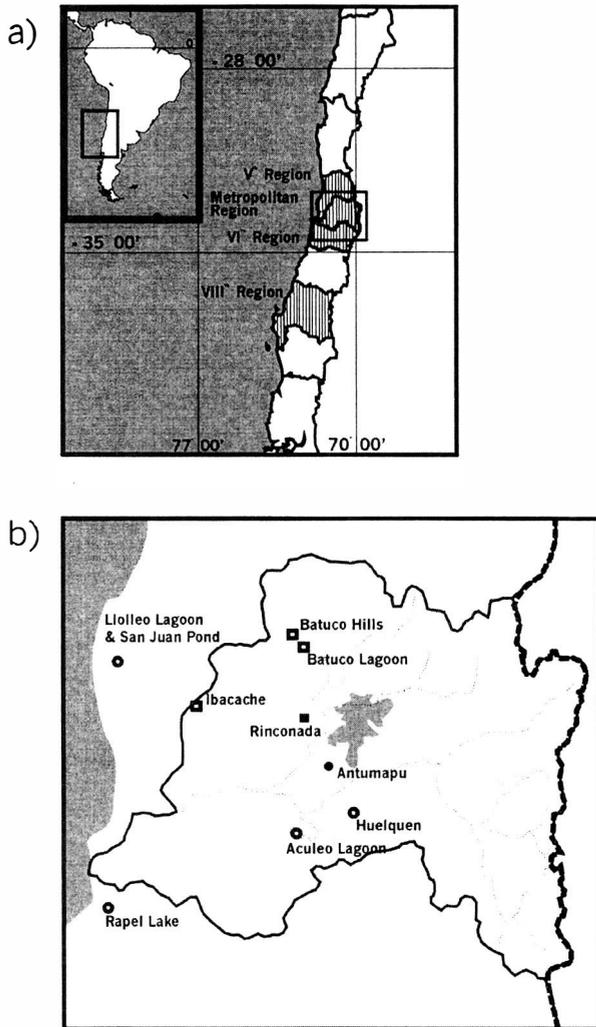


FIG. 1. The reported distribution of *Xenopus laevis* populations in Chile. (a) Vertically shaded areas show political regions of Chile reported to hold invasive populations of *X. laevis* (data from Lobos *et al.*, 1999; Hermosilla, 1994; and this study). (b) Enlargement of (a) showing sites of capture of *X. laevis* in this study (solid symbols), from Lobos *et al.* (1999, square symbols), and recently discovered localities (round symbols). Dotted lines represent major rivers, and the heavy dashed line an international boundary.

In the only published study of the ecology of *X. laevis* in Chile, Lobos *et al.* (1999) analysed the stomach contents of four populations of *X. laevis* in the Metropolitan Region of Chile, including two pristine habitats (Batuco Hills and Ibacache, see Fig. 1), between November of 1997 and February of 1998.

MATERIALS AND METHODS

STUDY SITES

The first locality, Antumapu – in the Pintana (33° 37'S, 70° 39' W), is an artificial pond on the Antumapu campus of the University of Chile. Stone walls form the bank to a depth of 1 m, with a total area of 400 m² (40 × 10 m). One third of the pond is overgrown with reeds (*Scirpus californicus*); the rest of the pond has open water with dense mats of algae and submerged alien pondweed, *Elodea canadensis*. The pond is fed and

drained by an irrigation channel that links with a number of other dams and ponds.

The second locality, Rinconada Dam (33° 29'S, 70° 54' W), is a large rain-fed dam with a southern wall of about 15 m and a flooded area of approximately 79 000 m². The dam feeds a large agricultural research station of the University of Chile at Rinconada. Emergent aquatic vegetation is poor (a few stands of *Cyperus* sp.), but submerged vegetation includes *Eragrostis* sp. and *Ludwigia peploides* at the dam wall. The water, during this study period, was highly eutrophicated with blooms of a blue-green alga (*Microcystis* sp.).

Environmental variables were measured at a depth of 0.15 m at 09.00 hr on the second visit to each locality. Water temperature was measured (to the nearest 0.5° C) with an alcohol thermometer, conductivity with a conductivity meter (model 72, ESD inc. Delaware, USA), dissolved O₂ by the Winkler method, and pH with a digital pH meter (EC310, HACH Colorado USA).

DENSITY ESTIMATES

Adults clawed frogs were captured using simple funnel traps (buckets with tight fitting lids, modified by lateral insertion of open cones) baited with liver. Five traps were set at dusk and collected at dawn at approximately two week intervals during March, April and May, 2001. All *X. laevis* caught were sexed using external morphological features (protruding labial lobes in females and formation of nuptial pads on the forearms of males). Animals were then freeze-branded with an individual three digit number (Daugherty, 1976) before being released at the site of capture (see Measey & Tinsley, 1998).

We assumed that this short winter period was not sufficient for significant changes to violate a closed population model (i.e. maturing sub-adults, deaths, im- and emigration), and thus we estimate population size using the Schnabel method, with 95% confidence limits calculated from a Poisson approximation to the Binomial distribution (Krebs, 1999). Density was calculated from the area of each site and its respective estimated population size.

DIET

Here we re-analyse stomach contents of 23 *X. laevis* collected from Antumapu (30 January 1998, Lobos *et al.*, 1999) by placing prey into groups by habitat and consequently by predation mechanism (Measey, 1998). In addition, stomach contents were taken from 21 *X. laevis* collected from Rinconada (10 March, 2001). In both cases, stomachs were removed from fixed animals and the contents enumerated and identified (see Lobos *et al.*, 1999).

RESULTS

A total of 27 adult *Xenopus laevis* was captured in funnel traps from the pond at Antumapu, with six individuals being recaptured during this study. Many *X. laevis* larvae at various developmental stages were ob-

served swimming and breathing at the water surface on each visit. The water temperature was noted as 23° C, pH was 8.6, dissolved O₂ 4.1 mg l⁻¹ and conductivity 617 µS. At Rinconada Dam, 1913 individual *X. laevis* were caught on all trapping occasions, of which 37 were recaptured. Numbers of *X. laevis* caught in the traps on 4 April far exceeded our expectations (after the low numbers caught at Antumapu) with three of the five traps having so many animals that all air in the trap was excluded, and consequently 323 animals drowned. These animals were returned to the laboratory and fixed. On subsequent trapping occasions at Rinconada, traps were checked on an hourly basis throughout the night, avoiding any further fatalities. No *X. laevis* larvae were observed at Rinconada. The water temperature was noted as 11° C, pH 8.8, dissolved O₂ 3.6 mg l⁻¹ and conductivity 129 µS.

DENSITY ESTIMATES

Proportions of males and females caught were significantly different, suggesting trapping bias. To account for this, we calculated population estimates for both sexes, and then recalculated population estimates using female mark-recapture data only (numbers shown in parentheses below). Table 1 shows the numbers of captured and recaptured *X. laevis* on each sampling occasion. Using the Schnabel method, the population estimate at Antumapu was 43 (95% confidence limits of 20.1 to 98.4) individuals (39.2 females, with 95% confidence limits of 18.3 to 89.9). Thus, density is calculated as 0.11 (females 0.10) clawed frogs m⁻². Observed movement of individuals (Lobos & Garín, 2002) leads us to believe that the open model of population estimate (150 individuals) would more accurately reflect the size of the population. However, low numbers of recaptures

TABLE 1. Capture recapture data for adult *Xenopus laevis* (numbers of males in parentheses) caught at an artificial pond on the Antumapu in the Pintana, Metropolitan Region, Chile; and Rinconada Dam, Metropolitan Region, Chile.

ANTUMAPU			
Date:	31.03.01	12.04.01	26.04.01
Day	1	13	27
Captured	6 (0)	22 (2)	5 (0)
1 st marked		3 (0)	2 (0)
2 nd marked			1 (0)
Released	6 (0)	22 (2)	5 (0)
RINCONADA			
Date:	04.04.01	20.04.01	08.05.01
Day	1	17	34
Captured	467 (131)	810 (178)	673 (86)
1 st marked		8 (2)	5 (0)
2 nd marked			24 (0)
Released	144 (38)	810 (178)	673 (86)

prevent a meaningful analysis using an open model. At Rinconada, the estimate of the population of *X. laevis*, using the Schnabel method, was estimated as 19 824 (95% confidence limits of 14 649.9 to 27 583.2) individuals (13 797 females, with 95% confidence limits of 10 200.8 to 20 059.6). Hence, for Rinconada, density can be calculated 0.25 (females 0.17) clawed frogs m⁻².

DIET

Table 2 shows the stomach contents of *Xenopus laevis* in Antumapu, consisting mainly of benthic elements (75%) – snails (*Physa* sp. 64.17%) and chironomid larvae (10.44%), both of which are tolerant of poorly oxygenated water. Zooplankton consisted solely of ostracods, which made up 17.91% of the total numbers of prey consumed.

Stomach contents of the 21 Rinconada *X. laevis* show the major component of the diet was zooplankton (95.24%), consisting of cladocerans (60.67%) and ostracods (35.57%). Other items were from benthic (3.07%), nektonic (0.27%) and terrestrial (1.41%) elements (Table 2).

DISCUSSION

The central area of Chile is one of five regions of the world to have a Mediterranean climate, along with the South African Cape and California (di Castri, 1991). In California, McCoid & Fritts (1995) reported the year-round growth of *Xenopus laevis* in what they described as 'optimal conditions', with an extended breeding season, and maturation in as little as eight months. This is considered a prime reason for the frog's rapid establishment and continuing expansion of range there (McCoid & Fritts, 1995). Here we discuss our results and the possible impacts of this invasive anuran in the Mediterranean region of Chile.

Density estimates recorded here show great variability and dependence on various food sources. It is unlikely that the population of *X. laevis* at Rinconada Dam remained closed *sensu stricto* over the trapping period; for example, many potential predators were observed at the dam (e.g. *Nycticorax nycticorax*, night heron; *Casmerodius alba*, great egret). However, we consider that any resulting fluctuation would not significantly change a population of this size, and hence we consider that the closed population estimate (19 824 individuals) is acceptable at this locality. The large number of animals found at Rinconada Dam is consistent with findings at Edwards Airforce Base, California (J. Crayon personal communication), but an order of magnitude greater than those estimated in South Wales (Measey & Tinsley, 1998). Rinconada Dam acts as a reservoir for surrounding farmland, which includes vineyards.

Sex ratios at both localities were found to be highly skewed in favour of females, which may be more attracted to the baited traps used in this study. Biased sex ratios have been previously observed in California and

TABLE 2. Analysis of prey items recovered from stomachs of 24 *Xenopus laevis* caught on 30 January 1998 at a pond on the Antumapu in the Pintana, Metropolitan Region, Chile; and 21 *Xenopus laevis* caught on 10 March 2001 at Rinconada Dam, Metropolitan Region, Chile.

ANTUMAPU						
Prey items	Total	% <i>X. laevis</i> eating (n=24)	Items ingested		% composition	
			mean	range		
Zoobenthos					74.61	
Diptera						
Chironomidae (larvae)	67	12	5.58	1-12	10.44	
Mollusca						
<i>Physa</i> sp.	412	20	20.60	3-33	64.17	
Zooplankton					17.91	
Crustacea						
Ostracoda	115	21	5.48	2 – 12	17.91	
Nekton					5.45	
Coleoptera						
Hydrophilidae	7	6	1.17	1 – 2	1.09	
Dytiscidae	5	2	2.50	2 – 3	0.78	
Odonata						
Libellulidae (larvae)	1	1	1.00		0.16	
Coenagrionidae (larvae)	16	9	1.78	1 – 3	2.49	
Aeshnidae (larvae)	4	2	2.00		0.62	
Hemiptera						
Corixidae	1	1	1.00		0.16	
<i>Notonecta</i> sp.	1	1	1.00		0.16	
Terrestrial					2.02	
Coenagrionidae	1	1	1.00		0.16	
Lepidoptera						
Noctuidae	6	6	1.00		0.93	
Dermaptera						
<i>Forficula</i> sp.	1	1	1.00		0.16	
Coleoptera						
Buprestidae	1	1	1.00		0.16	
Hymenoptera						
Formicidae	2	2	1.00		0.31	
Arachnida						
Araneae	1	4.17	1.00		0.16	
Crustacea						
Isopoda	1	4.17	1.00		0.16	

attributed to the trapping method, as use of seine nets did not reveal skewed ratios (J. Crayon personal communication). If both populations are considered to have equal proportions of males and females, then estimates made using only females could be doubled for total population estimates (i.e. including uncaught males). This would suggest even larger populations of *X. laevis* both at Antumapu (499) and Rinconada (39 647), as well as a doubling of density and impact.

Diet determined from stomach contents of animals captured at Antumapu showed a predominance of benthic invertebrates, as seen throughout the natural and non-natural range of *X. laevis* (Tinsley & McCoid, 1996; Measey, 1998). At Rinconada, the stomach con-

tents consisted mostly of zooplankton (cladocerans and ostracods) by number. However, this may give a false impression of contribution of prey to diet, as the mass of individually enumerated zoobenthos (present in all stomach contents) is greater than zooplankton (Table 2b). The large quantities of zooplankton ingested may change with seasonal abundance, as is known in other populations (Measey, 1998). Ostracods have previously been found to pass through the digestive system of clawed frogs unharmed (Measey, 1998).

Predatory impact on benthic species from *X. laevis* may have serious consequences for the biodiversity of Chile's macrobenthic invertebrates in lentic habitats. Absence of vertebrate prey items in these diet analyses

TABLE 2. (continued...)

RINCONADA					
Prey items	Total	% <i>X. laevis</i> eating (n=21)	Items ingested		% composition
			mean	range	
Zoobenthos					3.07
Mollusca					
<i>Physa</i> sp.	3	1	3.00		0.09
Diptera					
Chironomidae (larvae)	97	15	6.47	1 – 27	2.98
Zooplankton					95.24
Crustacea					
Cladocera	1944	21	92.57	5 – 500	59.67
Ostracoda	1159	21	55.19	8 – 295	35.57
Nekton					0.27
Coleoptera					
Dytiscidae	4	3	1.33	1 – 4	0.12
Hemiptera					
<i>Notonecta</i> sp.	5	2	2.50	1 – 4	0.15
Terrestrial					1.41
Arachnida					
Acarina	44	12	3.67	1 – 10	1.35
Araneae	2	2	1.00		0.06

cannot be taken to assume that such items are not eaten. Indeed, other alien populations of *X. laevis* have already been shown to prey upon small fish and amphibians (Lafferty & Page, 1997; Crayon, in press). At such high densities, it would seem inevitable that significant impact is made by predation alone; beyond this, exotic aquatic predators to natural water bodies may prompt trophic cascades, altering native species diversity and composition (Measey, 1998). Further secondary impacts may be associated with increased water turbidity and nutrient release, caused by *X. laevis* disturbing the sediment, and a change in population dynamics of native predators.

The Mediterranean region of central Chile has five native anuran species which are listed as vulnerable or endangered (Glade, 1983). Hunting and loss of habitat are presumed to have caused the disappearance of these species from much of their former range. However, like California and the UK, predation by clawed frogs on eggs, larvae and metamorphs of native amphibian species in Chile is an ongoing concern. Studies on the vulnerability of Chilean endemic amphibians, especially the mostly aquatic leptodactylid *Caudiverbera caudiverbera*, are urgently needed to assess the possible effects of the spread of alien invasive species.

Despite a history of 30 years of invasion by *X. laevis* in Chile, to date there have been no comprehensive studies documenting the impacts and spread of this species. Chile has a rapidly expanding viticulture industry that has the potential to take this invader, through extensive irrigation corridors, into new and previously

uncolonized areas. Concerns about the potential impacts of *X. laevis* introductions in the US have led to the outlawing of their possession in Arizona, California, Florida, Louisiana, Nevada and Utah (see Crayon, in press). Nevertheless, anecdotal evidence continues to mount concerning the direct predatory effects of this invasive anuran, and indirect effects (such as competition) have yet to be studied. In Chile the potential exists for the high densities of *Xenopus laevis* we demonstrate here to invade a large area of the country, especially when their presence in temperate UK habitats is considered. There is an urgent need for further study of the problems caused by *X. laevis* in Chile and throughout its introduced range.

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SHORT NOTES

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FAECAL COLLECTOR FOR FIELD STUDIES OF DIGESTIVE RESPONSES IN FOREST TORTOISES

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Key words: Chelonoidis denticulata, digestive responses, free-ranging, French Guiana

Over the years, many studies have been conducted on passage of digesta through the vertebrate gut, particularly in species considered to be important endozoochorous agents, i.e. species that disperse seeds in their faeces (Karasov *et al.*, 1986; Hume, Morgan & Kenagy, 1993; Martínez Del Rio & Restrepo, 1993). In endozoochorous seed dispersal, seed survival depends largely on how well the seeds are adapted to pass safely through the digestive tract. Gut passage is a delicate but necessary stage for seeds that are dispersed endozoochorously (Stevens & Hume, 1995). Many authors consider zoochory to be a means for seeds to (1) be deposited in microsites with physical and biological characteristics that increase the probability of successful germination and establishment (Augspurger, 1984; Howe, 1993a,b); (2) escape from natural predators (Schupp, 1988; Hulme, 1998; Hulme, Lins E Silva & Sousa, 2000); and (3) reduce intraspecific competition (Loiselle, 1990; Notman, Gorchov & Cornejo, 1996). In some plant species, seed passage through a dispersal agent's gut significantly increases the proportion of seeds germinating and/or their speed of germination (in chelonians: Braun & Brooks, 1987, Cobo & Andreu, 1988; in lizards: Willson *et al.*, 1996; in birds: e.g. Izhaki & Safriel, 1990; in mammals: e.g. Julliot & Sabatier, 1993).

The great majority of studies on digestive processes have been conducted on animals in captivity, under controlled conditions. These studies have shown that digestive processes, particularly digesta transit rate, depend on a variety of factors, such as temperature (Parmenter, 1981, Spencer, Thompson & Hume, 1998) and the composition and volume of digesta (Bjorndal, 1989; Clench & Mathias, 1992; Brand, Lanyon & Limpus, 1999). The combined effects of these factors make the understanding of how they affect the fate of seeds a complex matter (Bjorndal, 1991).

Most studies on endozoochory have two main objectives: (1) to determine the effects of gut passage on seed germination; and (2) to appreciate effects of scat deposit site on seedling establishment. To conduct such studies, it is necessary to collect animal faeces. How this is achieved mostly depends on the habitat of the animal that is studied. While it is relatively easy to collect faeces of animals living in open habitats, it is much more difficult in dense vegetation such as in tropical rain forests. In the latter, the success of faeces collection will depend largely on the type of animal studied. For instance, in some primate species it is relatively easy to detect droppings falling down from the canopy, especially when the animals defecate daily at fixed times and locations (Julliot, 1996). In contrast, it is much more difficult to find scats of more inconspicuous animals, such as tortoises, that live in dense vegetation at ground level.

The South American yellow-footed tortoise (*Chelonoidis denticulata*) is a typical species of South American tropical rain forests. It has a wide geographic range throughout northern South America, and is absent from only a few areas (e.g. Northern Colombia, Paraguay). On average, the linear length of the shell varies between 250 mm and 330 mm, and it weighs between 3.5 kg and 5.0 kg (Métrailler & Le Gratiet, 1996). During 15 months of field work at the Scientific Station of Nouragues (C.N.R.S. UPS 656; 4°05'N, 52°40'W) in French Guiana, I found no tortoise scats in the field, even when using a spool-and-line method to study movement patterns. Therefore, to obtain the faeces required for the study of their natural diet, tortoises were captured and confined individually in closed, plastic containers until they defecated (generally within 30 min), after which they were released. That study showed that *Chelonoidis denticulata* is a generalist, opportunistic feeder that feeds mainly on a variety of fruits. Thus, *C. denticulata* might be an important endozoochorous agent within neotropical rain forests.

For various reasons, "stress faeces" collected as described above cannot be used to determine parameters such as mean retention time, defecation rhythm and, especially, effects of gut passage on seed germination. For example, it is usually not known when the seeds found in such faeces were ingested, and seeds may not have undergone the whole action of natural gut passage. Thus, a faecal collector would be a useful device to easily obtain naturally deposited faeces in the field.

Bjorndal (1991), and Avery *et al.* (1993) used a semi-permanent device to collect all excreta voided by *Trachemys scripta*. Their apparatus consisted of a modified tube connector that was placed over the turtle's tail and either glued to the turtle's skin at the base of the tail with a silicone sealant, or held around the tail with a wire fitted around the tube and through two holes drilled in the carapace. A large latex finger cot was attached to collect all excreta, while spaces between the tubing and the skin and the shell of the turtle were sealed with silicone. In studies on tortoises, Bjorndal (1987, 1989) and Meienberger, Wallis & Nagy (1993) used cloth bags

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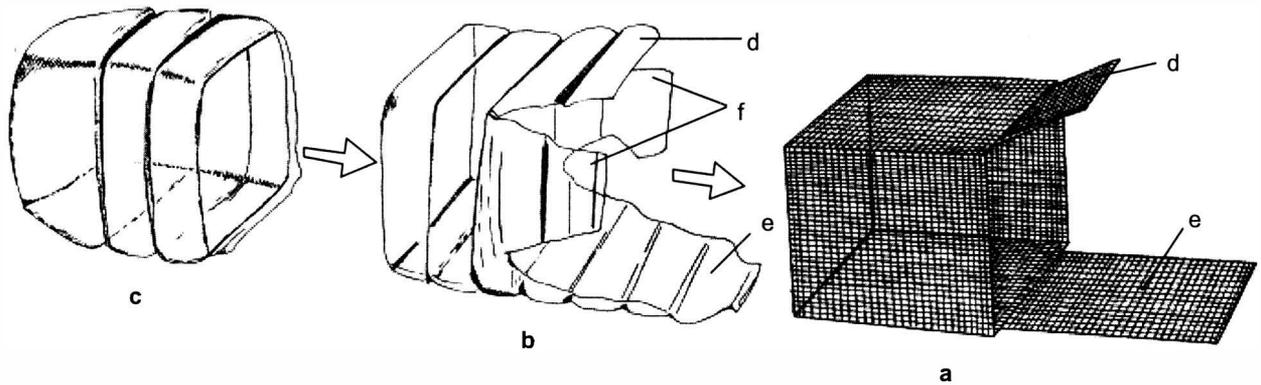


FIG. 1. View showing each part of the faecal collector separately: the mesh wire framework (a) with its two flaps, the plastic base (b) and the plastic cap (c).

that were attached to the animals by threads passed through holes drilled in the rear of the carapace. The animals were housed in individual pens where the ground was cleared of all objects that might tear the cloth bags. Other experiments with faecal collectors were conducted on animals housed in individual cages (Barboza, 1995; Hailey, 1998). All of these devices were designed to be used on animals maintained in captivity, i.e. in a simplified environment where most conditions were

controlled (Hamilton & Coe, 1982). All the latter techniques gave good results in their respective circumstances but were basically unsuitable for use in studies on free-ranging animals in complex environments. To enable the study of digestive processes in free-ranging tortoises, I designed a faecal collector that could be adapted to fit tortoises and that would withstand their normal negotiation of obstacles in their natural environment.

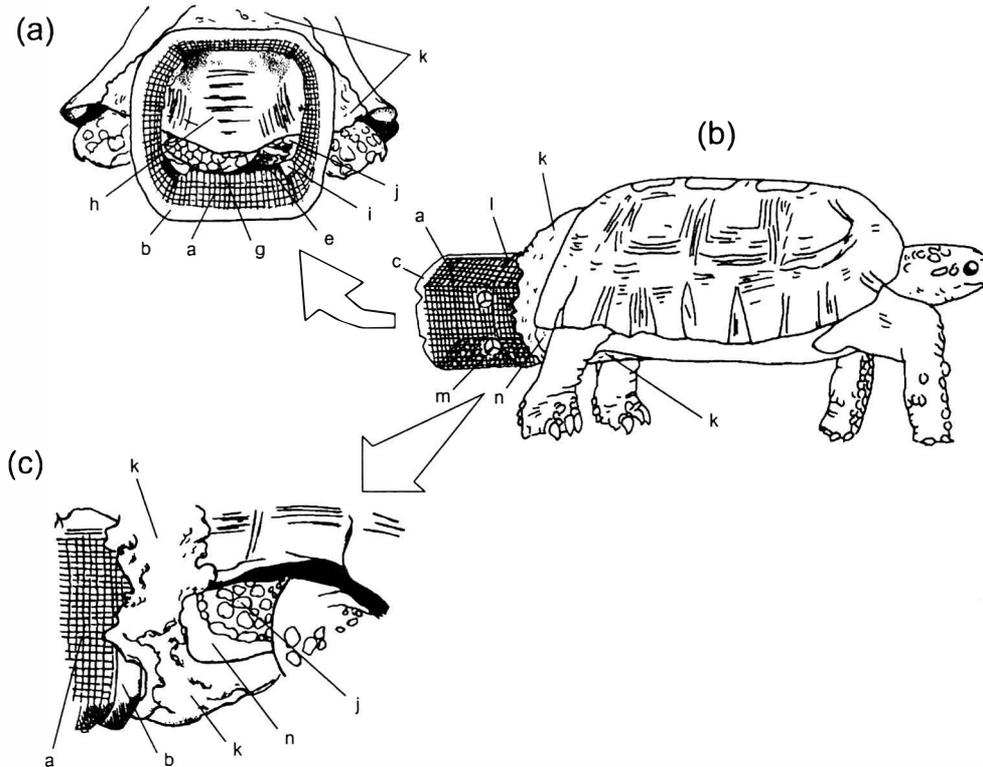


FIG. 2. (a) Back view showing the inside of the faecal collector without the removable cap. The tail is maintained naturally curved. The space between the shell and the plastron flap allowed the stretching of the tail along the plastron flap. (b) female equipped with a faecal collector. This device consisted of a wire-mesh framework attached to the rear of the carapace, with two plastic parts fitted around it and maintained with two bolts. (c) Details at the level of the hind foot. The framework and the plastic base were attached to the rear of the carapace with the Sintofer filler. Open spaces around the thighs were filled-in with a silicone sealant. No faecal matter could seep out. Key: a, mesh wire framework; b, plastic base; c, plastic cap; d, shell flap; e, plastron flap; f, lateral flaps; g, tail; h, supracaudal scute; i, space closed by the plastic base; j, thigh; k, Sintofer filler; l, plastic base limit under which the cap fitted into the base; m, bolt; n, silicone sealant (Drawings: S. Jouard).

The faecal collector consisted of three different parts: a wire-mesh framework and a soft, plastic casing in two parts (Fig. 1). The mesh framework formed a rectangular cage (with the lateral edges tied together with galvanized wire), 65 mm x 65 mm in cross section, 70 mm long, and open at both ends. Two flaps at one end were fitted tightly around the carapace and the plastron. The shape of the two flaps could be modified to fit exactly the shape of the shell, which varies between individuals. The flaps were 50 mm wide and either 40 mm long (carapace flap) or 80 mm long (plastron flap). The greater length of the plastron flap accommodated the more anterior position of the edge of the plastron and the concavity of the male plastron. The supracaudal scute of males is inwardly curved, reducing the angle of axial stretching of the tail; this required the plastron flap to be curved slightly, lowering the device a few centimetres towards the ground. As the female plastron is straight, the device was mounted level and continuous with the plastron (Fig. 2b).

The plastic casing was made from a 1.5-litre plastic bottle, square in cross section, in which drinking water was sold. The bottle was slightly larger in cross section than the mesh framework, and so fitted neatly around it. The bottle collected liquid faecal matter that might otherwise seep through the mesh, and also protected the collected faecal matter from rainfall. The base of one bottle was used as a removable cap that fitted over the bottomless, lower part of a second bottle (Fig. 1). The plastic casing had two flaps that were slightly shorter than those of the mesh framework (Fig. 1); the plastic flaps were drilled and attached to their corresponding mesh flaps with wire. The plastic casing was trimmed to fit the carapace closely, filling any gaps at the edges of the mesh frame. The base of the device had to stop at the level of the marginate scutes, in order not to hamper the locomotion of the tortoise (Fig. 2c). Thus, profiling the flaps demanded painstaking attention to detail. Once the plastic base was shaped, the device was attached to both shell and plastron with a two-component filler designed for repairing coachwork (Sintofer standard polyester filler, Sinto, Aubagne, France). Once dried, the filler stuck very well to the rough surface of the carapace, while both the casing and the frame were embedded tightly in the filler.

Finally, to avoid loss of faecal matter around the hind feet, open spaces were filled-in with a waterproof silicone sealant (Rubson silicone made by Henkel, France SA; Fig. 2c). Once dried, this material had to adhere well to the animal's skin, otherwise it might have fallen off and left a gap. Such an opening would have remained small while the animal was walking and in most other positions. Only if the tortoise retracted wholly into its shell (e.g. if danger threatened) would quite a large space have appeared; in the unlikely event of the collector being full, leakage might then occur. The removable cap facilitated quick retrieval of collected faeces. The cap was held in place by two parallel, transverse bolts

that prevented it from revolving around its axis and thus prevented leakage (Fig. 2b).

In tortoises, the tail is usually curled in the space between the carapace and the plastron (Fig. 2a) and is only extended when the animal defecates and during mating. The device was designed so that it would not disturb this extension. All wire-mesh edges that might have come in contact with the tortoise's skin were curved and coated in silicone to prevent injuries. After use, it was relatively easy to remove the device with a hammer and a chisel, without injuring the animal. Usually, the Sintofer filler could be removed all in one piece, leaving the carapace clean. A health inspection of the tortoises at the end of the experiment revealed no injuries caused by the device.

For use on sub-adult tortoises a smaller version of the faecal collector was made from a 0.75-litre plastic bottle and a wire mesh frame 42 mm long and 62.5 mm on each side. The two mesh flaps were 45 mm wide and 20 mm and 40 mm in length for the carapace flap and plastron flap, respectively.

Ten adult *C. denticulata* were fitted with the faecal collector, as described. The mean mass (\pm SD) of the collectors was 302 ± 34 g and the mean volume was 309 ± 53 ml, representing $8.1\pm 1.7\%$ of the body mass and $7.5\pm 1.1\%$ of the volume, respectively, of the equipped tortoises. Six faecal collectors were equipped with transmitters for radio-tracking. The mean mass (\pm SD) of the transmitters was approximately 85 ± 5 g and the whole equipment (collector + transmitter) weighed 401 ± 42 g ($n=6$), which was $9.9\pm 1.5\%$ of the body mass. The smaller collector fitted to a sub-adult had a mass of 175 g and volume of 164 ml, corresponding to 8.0% of the body mass and 9.7% of the volume of the tortoise, respectively.

During the present study, the Sintofer filler used to attach the collector to the tortoise's back was resistant to moisture and all other factors impinging on the device. Unlike previously described devices, this faecal collector did not suffer from forces of tearing or compression and none was torn away from the free-ranging animals over periods of 45 days.

As the plastic bottles were transparent, the presence of faeces could be detected easily, albeit with the aid of a flashlight when the device was dirty. Thus, tortoises could be checked several times a day to estimate defecation rhythm and digesta transit time, but with minimal disturbance. Faeces were removed, when detected, by unscrewing the bolts and removing the cap. In general, the retrieval of faeces took less than 10 min and could usually be done at the site where the tortoise was found. The time elapsed between consecutive defecations ranged from one day to more than 38 days and depended greatly on the activity level of the tortoise. Consequently, any stress induced by the retrieval of faeces was infrequent.

Once fitted, the collector (with its additional transmitter) did not protrude above or to the sides of the

carapace. Consequently, most normal movements of the animals were not hindered. However, the additional length at the back of the shell (73 ± 13 mm, $n=10$) might have hampered the animal when turning in very restricted places, such as tree-fall gaps, which are important as refuges. Moskovits (1985) showed that gaps represented half of all shelters used by *Chelonoidis denticulata*, whereas at Nouragues they represented on average 44% of the shelters used (Josseaume, 2002). As tree-fall gaps constituted 62% of all shelters used by tortoises equipped with the faecal collector in this study, it appears that the collector did not prevent use of this important refuge. In terms of home range, the great individual variability observed in many tortoise species (e.g. Stickel, 1950; Kiestler, Schwartz & Schwartz, 1982), including *Chelonoidis denticulata* (Moskovits, 1985; Josseaume, 2002), demands large sample sizes to compare tortoises with and without a faecal collector. At Nouragues, several individuals were followed using a spool-and-line method, or by radio-tracking, to study their displacement patterns. The mean home range (\pm SD) of tortoises without a faecal collector, determined by the minimum convex polygon method, was 5.4 ± 3.0 ha ($n=7$; minimum 2.3 ha; maximum 8.9 ha), whereas that of free-ranging individuals equipped with a faecal collector was 3.2 ± 2.5 ha ($n=6$; min: 0.9 ha; max: 7.7 ha). The difference between the two samples was not statistically significant (Mann-Whitney U -test: $U=32$, $P=0.116$). Foraging patterns and feeding rates of animals with and without faecal collectors appeared similar during direct observation of behaviour (Josseaume, 2002).

The carapace of tortoises constitutes a good support for devices such as the faecal collector described here. In general, these animals are both easy to handle and very sturdy. Therefore, a small additional mass does not affect their locomotion capacity (Zani & Claussen, 1994, 1995). For the researcher, this faecal collector is simple to set up and use. It is adjustable for the sex and the dimensions of the animal. The materials are easy to manipulate; the wire mesh is flexible, yet strong enough to resist both lateral and axial pressures resulting from the movements of a tortoise in cluttered parts of its habitat, such as tree-fall gaps.

In a study of the role of *Chelonoidis denticulata* as a seed disperser, the faecal collector permitted collection of seeds that had undergone the whole action of natural gut passage, in dense forest understory where it was practically impossible to find scats. This contributed greatly to the study of gut digesta passage and defecation patterns under natural conditions. The mean retention time in the field could be determined by feeding the tortoises with exogenous markers (i.e. non-indigenous seeds) just before they were released, while data on the daily displacements of the species could help in assessing the dispersal of tortoise-ingested seeds. Effects on ingested seeds could be studied with germination tests. Also, scat deposit sites, which may be important in the study of directional seed dispersal

(Howe & Smallwood, 1982), could be determined. This could be realized in an indirect way by combining the use of faecal collectors and radio-tracking.

In conclusion, the present faecal collector offers many new possibilities for the study of natural digestive responses in tortoises living in inaccessible habitats that are usually not amenable to this type of research.

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THE AMPHIBIAN FAUNA AT TWO ALTITUDES IN THE SINHARAJA RAINFOREST, SRI LANKA

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Rainforests, which typically harbour rich assemblages of amphibian species, are heterogeneous environments exhibiting habitat diversity in terms of vegetation structure and composition, moisture and temperature levels, and resource availability. In turn, these factors significantly affect the distribution of amphibian populations therein (Scott, 1976). Sri Lanka is a biologically diverse island with a rich complement of endemic plant and animal species. The high percentage of endemism has resulted in south-west Sri Lanka – where almost 90 % of the endemic vertebrates are concentrated (Erdelen, 1989; Senanayake *et al.* 1977) – being named as a biodiversity “hot spot” (Myers, 1990; Myers *et al.* 2000). Faunal inventories to date have revealed the presence of at least 53 species of amphibians in Sri Lanka (Dutta & Manamendra-Arachchi, 1996), of which 26 species are endemic to the island. It has been suggested more recently that the amphibian population in the country represents a far greater number of species than hitherto recognized (Pethiyagoda & Manamendra-Arachchi, 1998).

Despite the high proportion of endemic species in Sri Lanka, the amphibians have received very little attention from research scientists in the past. Rapid assessment by transect sampling in the forests of Sri Lanka to collect data on fauna and flora has provided limited information on the occurrence of amphibian species (IUCN & WCMC, 1997), while data on abundance are virtually absent.

We present here the results of the first quantitative study carried out to determine the abundance of amphibian species in the Sinharaja forest, which is the only relatively large, undisturbed, lowland rainforest on the island of Sri Lanka. Altitudinal variation in Sinharaja gives rise to two main habitat types – lowland and sub-montane forest. Therefore, we sampled sites at two different elevations so as to get a more complete idea of the diversity of species within this rainforest ecosystem than would have been possible had we restricted the study to a single site.

The Sinharaja forest is located in the south-western part of Sri Lanka, between latitudes 6°21' and 6°26' N and longitudes 80°21' and 80°38' E. It comprises the Sinharaja Reserve of 6130 ha and the Proposed Reserve of 5201 ha (IUCN, 1993), both of which are under state control. The Sinharaja forest was declared a World Heritage Site under the World Heritage Convention in 1989. The forest lies between the 3810 mm and 5080 mm isohyets, and rainfall is well distributed throughout the year, with no identifiable dry period (Gunatilleke & Gunatilleke, 1983).

Location 1: Kudawa. This site is located at the north-western end of the rainforest and is approached from the Kudawa village. Most of the area consists of parallel ridges and valleys, and in general the elevation does not exceed 400 m, with the exception of a few peaks. The vegetation here is classified as lowland wet evergreen forest, as originally described by De Rosayro (1950). The canopy of dominant trees reaches a height of around 40 m (Gunatilleke & Gunatilleke, 1983).

Location 2: Morning-side. Morning-side is situated at the eastern side of the Sinharaja forest at an elevation of around 900 m. The land here is relatively flat and is covered by sub-montane evergreen forest. The vegetation is essentially transitional, being intermediate in structure and physiognomy between the lowland wet evergreen and tropical montane forest types (IUCN, 1993). The height of the canopy trees is considerably lower than at Kudawa, and the Thangamalai plain at the extreme east has stunted vegetation and grasslands. The latter area was added to the Sinharaja Reserve only in 1988, and the boundaries have not been clearly demarcated as yet. Some areas contiguous with the Sinharaja forest, and proposed for inclusion within it, are still under private ownership (IUCN, 1993).

A population census of amphibians was conducted during the period April 1997 to April 1998 in the two selected locations within the Sinharaja forest. In total, forty-five quadrats were surveyed at each of the two study sites. Each quadrat measured 8 m x 8 m. The placement of the quadrats at the two sites was intended to represent a stratified random sample, inasmuch as the numbers of quadrats located by streams and within the drier areas of the forest were similar at both sites. Quadrats were not located in grassland areas at the edge of the forest at Morning-side. Sampling was undertaken monthly, with at least two quadrats being investigated at each site in each month. No sampling was conducted during days of heavy rain.

The technique used was the Visual Encounter Survey, a standard technique which has been recommended for the study of amphibian populations in tropical ecosystems (Heyer *et al.* 1994). The quadrats were systematically searched by walking in parallel paths across the plot, thoroughly searching among the litter, logs, rocks and vegetation up to a height of 3 m. Sampling was carried out at night when most species of amphibians are active. For all quadrats, a field crew comprising four persons made searches lasting one hour per quad-

rat, and sampling was carried out between 1900-2400 hr, using headlamps and torches. All the amphibians we captured were collected in bottles and were identified and released at the same locations the following morning. Collection of the animals enabled accurate identification and avoided double counting. Dipnets were used to sample amphibian populations in streams and pools.

TABLE 1. The numbers of amphibians captured in forty-five 64 m² quadrats at each of two study sites, Kudawa and Morning-side, in the Sinharaja rain forest, Sri Lanka. *Endemic species; ** Endemic genus. ¹ *Adenomus kelaartii* (Manamendra-Arachchi & Pethiyagoda, 1998) was previously named as *Bufo kelaartii* (Dutta & Manamendra-Arachchi, 1996). ² Bufonids with a small tympanum (less than one third of eye diameter) were earlier classified as *Bufo microtympanum* (Dutta & Manamendra-Arachchi, 1996) but now reclassified as *B. noellerti* (Manamendra-Arachchi & Pethiyagoda, 1998). ³ Species described by Manamendra-Arachchi & Gabadage, 1996. ⁴ An endemic rhacophorid, *Theoderma schmarda*, was observed opportunistically at Kudawa.

Species	Kudawa	Morning-side
Bufo		
<i>Adenomus kelaartii</i> ** ¹	9	43
<i>Bufo noellerti</i> * ²	-	3
<i>B. kotagamai</i> *	10	-
<i>B. melanostictus</i>	2	-
Rana		
<i>Rana aurantiaca</i>	20	123
<i>R. temporalis</i>	10	54
<i>Limnonectes corrugatus</i> *	3	27
<i>L. kirtisinghei</i> * ³	1	3
<i>Nannophrys ceylonensis</i> **	4	-
<i>Euphlyctis cyanophlyctis</i>	1	-
Rhacophoridae ⁴		
<i>Philautus nasutus</i> *	1	-
<i>P. variabilis</i>	11	90
<i>P. leucorhinus</i>	11	9
<i>P. temporalis</i> *	2	-
<i>P. hypomelas</i> *	2	-
<i>Rhacophorus microtympanum</i> *	11	48
<i>R. reticulatus</i> *	2	5
<i>R. macropus</i> *	31	90
<i>R. cavirostris</i> *	8	3
<i>Polypedates cruciger</i> *	4	3
<i>P. maculatus</i>	16	60
<i>P. eques</i> *	1	1
<i>P. longinasus</i> *	2	1
Microhylidae		
<i>Microhyla karunaratnei</i> *	-	8
<i>Ramanella obscura</i> *	-	3
<i>Kaloula taprobanica</i>	1	-
Ichthyophidae		
<i>Ichthyophis glutinosus</i> *	1	1
Total	164	575

Litter depth was measured at 25 randomly selected locations in each study site.

A total of 739 individuals of 27 species was recorded from the two study sites, including 19 endemic species – two belonging to endemic genera (Table 1). The number of species far exceeds the total number of species previously recorded in this rainforest (Fernando & Perera, 1998; IUCN & WCMC, 1997). Kudawa (lowland site), with 24 species, had a greater species richness than Morning-side, which had 19 species. Although the majority of the species were recorded from both sites at varying abundance, the following 11 species were recorded from only one of the two sites: *Bufo kotagamai*, *B. melanostictus*, *Nannophrys ceylonensis*, *Euphlyctis cyanophlyctis*, *Philautus nasutus*, *P. temporalis*, *P. hypomelas* and *Kaloula taprobanica* from Kudawa, and *Bufo noellerti*, *Microhyla karunaratnei* and *Ramanella obscura* from Morning-side.

In general, the number of individuals of most species was rather low, with as many as 17 species, 14 of which are endemic, represented by no more than ten individuals at either site. Although Morning-side had a lower diversity in terms of species, the abundance of most of those species that were present in both sites was greater at Morning-side than at Kudawa. Nine of the species recorded from both the sites were represented by at least ten individuals at one or both of the sites. Eight of these species (*Adenomus kelaartii*, *Rana aurantiaca*, *R. temporalis*, *Limnonectes corrugatus*, *Philautus variabilis*, *Rhacophorus microtympanum*, *R. macropus* and *Polypedates maculatus*) had higher numbers of individuals at Morning-side than at Kudawa. We recorded significantly higher numbers of bufonids, ranids, rhacophorids and microhylids at Morning-side than at Kudawa. It is noteworthy that only one caecilian, *Ichthyophis glutinosus*, was recorded from each of the two sites; this, however, may have been because the sampling regime was not geared to sample such deep-burrowing species. The depth of litter at Morning-side (mean±SE: 52±1.0 mm) was much greater than at Kudawa (13±1.5 mm).

Our results indicate that the amphibian fauna was richer in species but poorer in abundance in the lowland site than in the sub-montane site within the Sinharaja rainforest. In our survey, eight species were restricted to the lowland site while only three species were restricted to the upland site. Surveys carried out at three higher-elevation forests in Sri Lanka (above 1500 m) recorded fewer species than we found, providing evidence that species richness declines with altitude (Bambaradeniya & Ranawana, 1998).

A number of factors may contribute to the differences between the amphibian assemblages. For example, it has been reported that, in contrast to Amazonian species, most south-east Asian amphibian assemblages are riparian or develop in water, with only a few species developing terrestrially (Zimmerman & Simberloff, 1996). At Morning-side there are flat areas of grassland where permanent and semi-permanent pools

provide suitable breeding habitats for many species of amphibian, as compared to the fast flowing streams at Kudawa. Morning-side also has several man-made, aquatic micro-habitats in the form of abandoned pits from illegal gem-mining in the past (IUCN, 1993); the pits have now become breeding sites for many species (Fernando & Perera, 1998). It has been found elsewhere that as the number of pools in a habitat increases, so the probability of an amphibian species occupying that habitat also increases (Mann *et al.*, 1991; Vos & Stumpel, 1996). It is likely that the higher abundance of species requiring water to breed (e.g. *Rana aurantiaca*) observed at Morning-side primarily reflects the availability of pools.

Terrestrial habitat structure is also an important factor that influences the occurrence of amphibians within an ecosystem (Dupuis *et al.*, 1995; Morrison *et al.*, 1995). The vegetation structure and composition at our two study sites are strikingly different. The vegetation of the north-western part of Sinharaja, at the lower elevation zone, is dominated by large trees belonging to the family Dipterocarpaceae (Gunatilleke & Gunatilleke, 1983). The forest has a closed canopy, trees are tall and of large girth, and undergrowth is sparse. Morning-side, in contrast, has a relatively open canopy and a relatively dense understorey. It has been reported that tropical forest treefrogs usually prefer to perch on understorey vegetation, which seldom exceeds 3 m in height, and that only a few species occupy or forage in large canopy trees because of their intolerance of desiccation (Stewart & Pough, 1983). The rhacophorids at Morning-side were seen to forage preferentially among the leaves of *Pandanus* sp., a common shrub in the understorey at this site. Thus, the vegetation structure of the two sites may have been a significant factor in accounting for the differences in distribution of rhacophorids at the two sites. An increase in depth of the litter layer with increasing altitude has been noted previously (Scott, 1976; Woods & Gallegos, 1970), and the greater depth of litter at Morning-side may account, in part, for the greater abundance of bufonids, ranids and microhylids inhabiting the upland site.

The findings of this study have important implications for conservation policy. With regard to Sinharaja itself, the inclusion of the eastern sector within the reserve forest is seen to provide a boost to the survival of the amphibian species that prefer cooler temperatures and high levels of moisture. This area was included within the protective framework of the Sinharaja forest only in 1988 and a part of the forest still remains under private ownership. A large extent of this area had been severely degraded by illegal gem mining, cultivation and deforestation in the past, and the peripheral areas of the forest still continue to be used for the cultivation of cardamom. Interestingly, the abundance of gem pits may have facilitated breeding by some species. Since the area was included within the reserve many illegal activities have ceased, resulting in the regeneration of the forest. Because of the importance of adjacent peripheral habi-

tats for breeding of forest-dwelling species, conservation efforts should not be limited to the forest habitats but should be extended to the surrounding areas that could be said to form an integral part of the forest ecosystem.

The Kudawa section of the forest has received considerable protection over many years. Despite the high degree of protection, it is surprising to note that many species, especially those that were restricted to this site, were found in low numbers. This warrants further investigations to identify the ecological needs of these species and to determine whether the current conservation measures are adequate to maintain viable populations.

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**DIET OF *THELOTORNIS KIRTLANDII*
(SERPENTES: COLUBRIDAE:
DISPHOLIDINI) FROM SOUTHERN
NIGERIA**

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Thelotornis kirtlandii is an arboreal, venomous colubrid snake with a wide distribution ranging from the islands of the Bijagos Archipelago, Guinea Bissau; east through forested areas of west Africa and the Congo basin to Uganda and southern Sudan; south to northern Angola, north-western Zambia and south-central Tanzania (for review, see Broadley, 2001). It is typically a forest species (Broadley, 2001), and in southern Nigeria may attain high population densities in mangrove habitats (Luiselli & Akani, 2002).

Although the diet of its savanna congener, *Thelotornis capensis*, is well known (Shine *et al.*, 1996), few data are available on the food habits of *T. kirtlandii*, possibly due to its elusive habits and relatively inaccessible rainforest and mangrove habitats. Apart for general comments in books (e.g., "this snake feeds on lizards, birds, etc.", e.g. see Phelps, 1981), there are very few precise dietary records in the literature: this snake was found to prey upon nestling birds (*Spermophaga ruficapilla*), agamids (*Acanthocercus atricollis*), and scincids (*Mabuya maculilabris*) in the Democratic Republic of Congo (Loveridge, 1942); upon lacertids (*Ichnotropis capensis*) in Zambia (Broadley, 1991); and upon colubrid snakes (*Philothamnus carinatus*) in Zaire DCR (Bogert, 1940). From a swamp-rainforest of south-eastern Nigeria, Luiselli *et al.* (1998) recorded eight food items: one small bird (*Cisticola galactotes*) and seven geckos (*Hemidactylus fasciatus*). Here we present a detailed account of the diet of free-ranging *T. kirtlandii* from a region situated within the continuous Guinea-Congo rainforest belt (i.e. southern Nigeria, West Africa), with an analysis of prey-size predator-size relationships.

Our field study was carried out from September 1996 to May 2002 (with additional data recorded in 1994 and 1995), in some localities of south-eastern Nigeria (for

the territories surveyed cf. Luiselli & Angelici, 2000), situated in the eastern axis of the Niger Delta (Bayelsa and Rivers States), in Anambra, Akwa-Ibom, Abia and Cross River States. The study region is tropical, with a wet season from May to September and a dry season from October to April. The wettest period of the year is June-July, and the driest period between late December and February.

Methods used to survey the study area, capture snakes and analyse their food items, are detailed elsewhere (e.g. Luiselli *et al.*, 1998, 2002). Fieldwork was conducted under all climatic conditions, but with a bias toward diurnal hours (from 0800-1800 hrs) due to security constraints related to the prevailing unstable political situation. Field effort was almost identical in the wet (421 field days) and dry (418 field days) seasons.

We searched for snakes along standardized routes in the various microhabitats frequented by snakes at the study areas. We captured snakes by hand, but additional free-ranging specimens were captured by pitfall traps with drift fences and by traps used by locals to capture terrestrial animals. We always recorded the site of capture and the habitat at each capture site. Each snake was measured for snout-vent length (SVL, to the nearest 0.1 cm), weighed with an electronic balance, and individually marked by ventral scale clipping for future identification. Then, the snakes were palpated in the abdomen until regurgitation of ingested food or defecation occurred. In addition, specimens found already dead during our surveys (e.g. snakes killed by farmers, or by cars, etc) were dissected to determine if prey were present. We identified prey items to the lowest taxonomic level possible. We estimated mass of prey items at the time of its ingestion, when possible, by comparing the item to intact conspecifics of various sizes from our own personal collection, or measuring the fresh biomass in perfectly preserved items. This was not possible in faecal samples, which generally consisted of scales of reptiles. Although in this article we used data collected from both stomachs and faeces, specimens for which stomach contents were used were not generally used for faecal pellets, to avoid multiple counts of the same food items. However, the food contents from both faeces and stomachs of a same snake specimen were considered in the cases in which, for instance, faecal samples contained mammal hair and the stomach a gecko).

Vouchers (of both prey and predators) were deposited in the herpetological collections of the Rivers State University of Science and Technology (Port Harcourt, Nigeria), of the Department of Agricultural Sciences of Uyo University (Uyo, Nigeria), of the Faculty of Sciences of the University of Calabar (Calabar, Nigeria), of the Ecology Lab at F.I.Z.V. (Rome, Italy), and of the Cross River National Park (collections in both Akamkpa and Butatong, Nigeria).

All statistical tests were two-tailed, with alpha set at 0.05. Means are followed by ± 1 SD.

A total of 133 specimens (44 smaller than 50 cm total length – assumed to be subadults in this study – and 89 larger than 50 cm total length) were examined for the present study (see Appendix 1 for a list of the localities of capture). Maximum size was 122.5 cm for males, and 131.4 cm for females. When only specimens >50 cm in length are considered, males and females were similar in terms of mean length (males: 89.8±17.4 cm, $n=39$; females: 91.2±18.3 cm, $n=41$; differences between two samples: one-way ANOVA $F_{1,77}=2.384$, $P>0.3$).

A list of prey items is presented in Table 1. Based on this table, it is evident that 100% of the dietary spectrum of specimens >50 cm in length consisted of vertebrates, whereas a very few arthropod items were found in juveniles. It is not known whether these arthropod remains were primary feeding records, or secondary ingestion following the complete digestion of the original predator, e.g. a gecko or amphibian. It is likely that the second option would be more likely, as (1) specimens of *Thelotornis kirtlandii* kept in captivity never fed upon invertebrates; (2) these prey items were almost completely digested; and (3) there are no records supporting primary arthropod feeding by *Thelotornis* (e.g. see Shine *et al.*, 1996). Amongst vertebrates, nearly all the prey items were reptiles, with a single case of avian prey (the same record was presented in Luiselli *et al.*, 1998) and a single case of amphibian prey. Amongst reptiles, snakes were rarely eaten, whereas lizards – and particularly gekkonids – formed the main prey.

Female body size significantly positively influenced the size of the prey eaten (Spearman's $r^2=0.312$, ANOVA: $F_{1,18}=6.882$, $P=0.0189$), whereas the same relationship was not found in males ($r^2=0.103$, ANOVA: $F_{1,14}=0.108$, $P=0.594$). The slopes of these two regres-

sions differed significantly (heterogeneity of slopes test: $F_{1,30}=4.139$, $P=0.026$).

To the best of our knowledge, there are no other scientific reports on the diet of a large number of free-ranging specimens of *T. kirtlandii* from a single geographic region (although Shine *et al.*, 1996, presented a very detailed dietary study on the savannah congener *T. capensis*). Thus, it may be assumed that the present study, being the largest of its type available for *T. kirtlandii*, may have permitted finer (and perhaps different) conclusions from those already available in the literature. Nonetheless, at least in general terms, this study confirms previous literature suggesting that lizards account for a remarkable portion of the diet of free-ranging *T. kirtlandii*. It is interesting, however, that no chameleons were eaten by these snakes, although they were regularly found in congeners from elsewhere (e.g. *T. capensis*, see Shine *et al.*, 1996; *T. mossambicanus*, D. Broadley, *pers. comm.*). It perhaps reflects a global scarcity of these potential prey in the rainforest habitats of southern Nigeria (Akani *et al.*, 2002). On the contrary, gekkonids (and to a lesser extent also Scincidae) obviously represented the primary prey sources for both adult and subadult snakes. Our data also demonstrate that rainforest *T. kirtlandii* do not have as wide a dietary spectrum as their savannah congeners (Shine *et al.*, 1996), which are known to forage frequently also on amphibians (especially *Breviceps*, but including *Chiromantis xerampelina*) and on large lizards like *Acanthocercus atricollis* and *Gerrhosaurus* spp., in addition to snakes and mammals) (D. Broadley, *pers. comm.*). Amphibian and snake prey were extremely rare in forest *T. kirtlandii* from Nigeria. However, it is noteworthy that Lawson (1993) recorded

TABLE 1. Compilation of the original data available on food items of *Thelotornis kirtlandii* from south-eastern Nigeria.

Prey	Numbers of prey items in specimens <50 cm length	Numbers of prey items in specimens >50 cm length	Cumulative number of prey items
Invertebrates			
Mantodea	2	0	2
Birds			
<i>Cisticola galactotes</i>	0	1	1
Reptiles			
<i>Gastropyxis smaragdina</i>	0	1	1
<i>Philothamnus</i> sp.	0	2	2
<i>Mabuya</i> sp.	7	11	18
<i>Panaspis</i> sp.	1	0	1
<i>Agama agama</i>	4	7	11
<i>Hemidactylus fasciatus</i>	2	8	10
<i>Lygodactylus</i> sp.	2	6	8
Undetermined Gekkonidae	3	4	7
Amphibians			
<i>Phrynobatrachus</i> sp.	0	1	1
TOTAL	21	41	62

a case of predation by *T. kirtlandii* on *Phrynobatrachus auritus* in Korup National Park (western Cameroon, close to the border with Nigeria). According to Don Broadley's unpublished data (*pers. comm.*), geckos represented in the diet of savanna species were mostly *Lygodactylus*, but there was one *Hemidactylus platycephalus*. In our case, both *Hemidactylus* and *Lygodactylus* spp. were frequently preyed upon. It is noteworthy that there was an apparent conflict in our data of a diurnal snake feeding on a nocturnal gecko – e.g. *Hemidactylus fasciatus*. However, these geckos are often found during the daytime at the mouths of tree holes inside the shady forest, and we suppose that *T. kirtlandii* may ambush them in these situations, or – alternatively – they may have a specific hunting strategy that allows them to locate nocturnal geckos in their retreats.

In general, it is likely that these differences between *T. kirtlandii* and *T. capensis* may reflect local variations in prey resource availability (due to different habitat structure and geographic regions) rather than species-specific, genetically-encoded, divergence in foraging habits.

As for the prey-size/predator-size relationships, it is noteworthy (but as yet inexplicable) that the two sexes exhibited very different patterns, with the females tending to prey on larger organisms in relation to increases in their own body size, whereas the males which did not. It is possible that these diverging patterns may be linked to the different energy requirements of the two sexes for reproduction to take place, but supporting data are needed before this hypothesis can be tested.

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APPENDIX 1

Gazetteer of the localities of capture of *Thelotornis kirtlandii*. When possible, geographic coordinates taken by GPS "Garmin 12" are reported. When geographic coordinates were not taken, the site of capture is made available to further readers by citing its Local Government Area, or the main town close to it.

ABIA STATE: Aba; AKWA-IBOM STATE: Eket (04°50'N, 07°59'E); Stubbs Creek Reserve; Ifon (Etinang Local Government Area); BAYELSA STATE: Nembe (Nelga Local Government Area); Dagbairi (Sagbama Local Government Area); Toru-Ebeni (Mein/Oakiri Local Government Area); Sangana (Brass Local Government Area); Otukpoti (Ogbia Local Government Area); CROSS RIVER STATE: Ikpan Forest (08°38'N, 05°11'E); Okwangwo village; Obubra (06°08'11"N, 05°40'12"E); DELTA STATE: Abraka (banks of Ethiope River); Okuovu (Sapele Local Government Area); Patani; Ughelli (Isoko North Local Government Area); ONDO STATE: Osse River (06°58'13"N, 05°40'08"E); RIVERS STATE: Otari (04°53'N, 06°41'E); Tombia mangrove (04°46'51"N, 06°51'54"E); Orashi River (04°44'43"N, 06°38'10"E); Peterside (04°29'N, 07°10'E); Bonny Island (04°25'N, 07°15'E); Abonnema (Akulga Local Government Area); Ke (Degema Local Government Area); Kula (Degema Local Government Area); Ngo (Andoni Local Government Area); Okrika (Walga Local Government Area); Odiokwu (05°06'N, 06°37'E); Bikkiri (04°46'N, 06°36'E); Egita (05°15'N, 06°41'E); Awarra (05°21'14"N, 06°49'02"E).

BOOK REVIEW

Tadpoles of South-eastern Australia. A Guide with Keys. Marion Anstis (2002). 281 pp. Reed New Holland, Sydney. £29.99 (cloth).

This is a remarkable and extraordinary book. Marion Anstis has found and reared to metamorphosis the tadpoles of 84 frog species found in Tasmania, Victoria and New South Wales. She has drawn and photographed them all and has used this mass of information to construct keys so that any tadpole in this corner of Australia can be identified as to species. This is a unique achievement which could, and should, be emulated in other parts of the world.

The rationale for this massive enterprise, to which Marion Anstis has devoted much of her life, is that, while adult frogs are often difficult to find, both because of their secretive habits and as a result of their declining abundance, their tadpoles can often still be found. In these days when the definition of species is increasingly based on biochemical characters, requiring expensive laboratory procedures not available to field naturalists, it is somehow reassuring to be reminded of the value of careful anatomical observation and description.

Part 1 of the book provides background information, on frog taxonomy, development, tadpole collection and rearing, and conservation issues. The tadpole key, with a detailed account of the characters used to construct it,

makes up Part 2, which also includes smaller keys to eggs and embryos. Part 3 consists of full accounts of all 84 species; these each include a photograph of the adult, a distribution map, a drawing and photograph of the tadpole and a detailed drawing of the tadpole's mouthparts. The quality of the drawings and photographs is very high throughout. The species accounts also summarize what is known about the natural history, ecology and behaviour of each species.

Whether or not this book becomes a model for similar efforts elsewhere in the world, or whether it becomes an oddity in the herpetological literature, depends largely on how prepared biologists are to invest in the amount of effort that work like this requires. While the task that Marion Anstis has undertaken is a massive one, it pales into insignificance beside what would be required to carry out a similar study in many of the world's tropical forests, where amphibian diversity is so much greater. Sadly, such an enterprise is unlikely ever to be undertaken, for two reasons. First, this is not the kind of research that is ever likely to receive funding from conventional sources. Secondly, it would be overtaken by events; such is the rate of decline in amphibian diversity throughout the world, anyone attempting a project of this kind would very likely find that many species had become extinct before they had finished.

Tim Halliday
The Open University

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