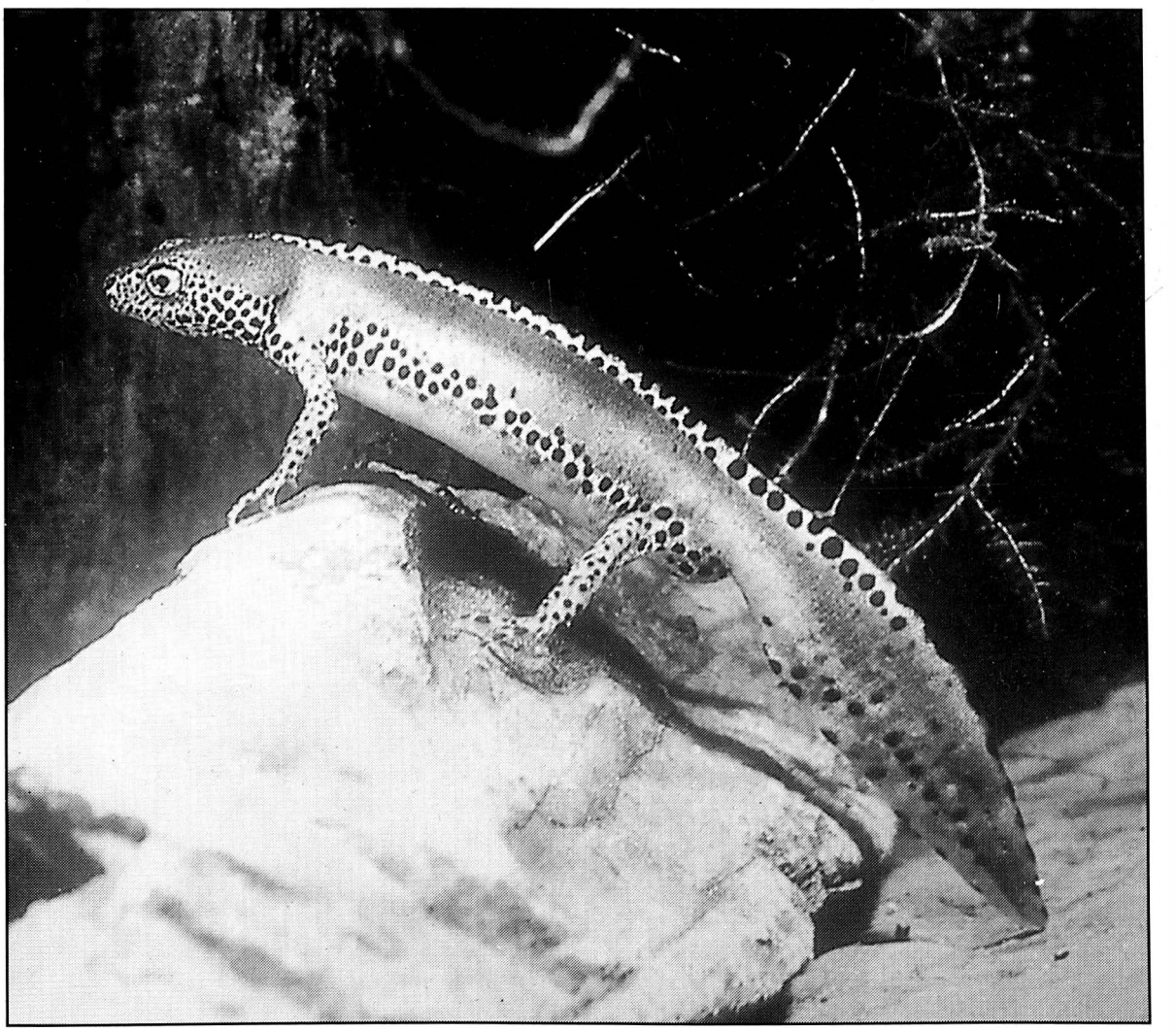
Volume 11, Number 1

January 2001
ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

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

Applications to purchase copies and/or for details of membership should be made to the Hon. Secretary, British Herpetological Society, The Zoological Society of London, Regent's Park, London NW1 4RY, UK.

Instructions to authors are printed inside the back cover. All contributions should be addressed to the Scientific Editor (address below).

BRITISH HERPETOLOGICAL SOCIETY

FRONT COVER: Male alpine newt, *Triturus alpestris apuanus* (M. Sparreboom)

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 1-8 (2001)

MORPHOLOGICAL DIFFERENTIATION OF THE ALPINE NEWT (*TRITURUS ALPESTRIS*) IN THE BALKANS: TAXONOMIC IMPLICATIONS

KONSTANTINOS SOTIROPOULOS[1], LJILJANA TOMOVIC[2], GEORG DZUKIC[3] AND MILOS L. KALEZIC[2]

[1]*Zoological Museum, Department of Biology, University of Athens, Athens, Greece*

[2]*Institute of Zoology, Faculty of Biology, Studentski trg 16, 11000 Belgrade, F. R. Yugoslavia*

[3]*Institute for Biological Research "Sinisa Stankovic", 29 Novembra 142, 11000 Belgrade, F. R. Yugoslavia*

A comprehensive survey of the Balkan alpine newt was undertaken to describe morphological differentiation between populations, and to test the validity of previously described subspecies. Thirty population samples derived from the major part of the Balkans, excluding Bulgaria and Albania, were studied for patterns of both morphometric and qualitative trait variability. On the basis of morphology, separate taxonomic status cannot be allocated to any of the currently recognized Balkan subspecies, with the exception of the southernmost (*T. a. veluchiensis*). Paedogenesis affected morphological variability significantly. Variability among paedotypic populations was found to be lower than intrapopulation variability of metamorphosed individuals.

Key words: Triturus alpestris, morphological differentiation, taxonomy, paedogenesis, Balkan Peninsula

INTRODUCTION

Triturus alpestris (Laurenti, 1768), is a fairly widely distributed European newt species. Its range extends from north-east France to western Ukraine, and from southern Denmark to northern Italy and the Balkans, as far as the northern Peloponnese. Isolated parts of its range exist in northern and central Spain, as well as in southern and central Italy (Griffiths, 1996).

The alpine newt is a highly polytypic species. The status and nomenclature of the alpine newt subspecies have a confused taxonomic history lasting more than a century. Thirteen subspecies have been described, mainly according to external morphological features (head size and shape in particular) and coloration pattern. Currently, various subspecies are considered to be valid (see Griffiths, 1996 and Zuiderwijk, 1997 for the most recent accounts). The nominotypical subspecies *T. a. alpestris* is by far the most widely distributed. The subspecies *T. a. cyreni* Wolterstorff, 1932 is confined to northern and central Spain; *T. a. inexpectatus* Dubois & Breuil, 1983, to central Italy and Calabria; while the range of *T. a. apuanus* (Bonaparte, 1839) includes south-eastern France and central-northern Italy.

The Balkan Peninsula is supposed to be the centre of alpine newt radiation (Arano & Arntzen, 1987; but see Herrero *et al.*, 1989). Moreover, the Balkans have been considered to be a hot spot of alpine newt evolutionary diversification due to the description of numerous subspecies. In the western and central Balkans, subspecific status has been erected for a number of isolated populations exhibiting paedogenesis (*sensu* Reilly *et al.*, 1997) which inhabit high-mountain glacial lakes. These are: *T. a. reiseri* (Werner, 1902) – Prokosko lake (Bosnia); *T. a. montenegrinus* Radovanovic, 1951 - Bukumirsko lake (Montenegro); *T. a. piperianus* Radovanovic, 1961 - Kapetanovo and Manito lake (Montenegro); and *T. a. serdarus* Radovanovic, 1961 - Zminicko lake (Montenegro). Among Balkan alpine newt subspecies, *T. a. veluchiensis* Wolterstorff, 1935 exhibits the widest distribution. It has been proposed to be the only alpine newt in Greece (Breuil & Parent, 1988; Sotiropoulos *et al.*, 1995) while its distribution extends to the south-eastern parts of Albania (Bruno, 1989).

The taxonomic status of Balkan subspecies is still uncertain. There are discrepancies in results obtained by limited morphological study (Ernst, 1952; Rocek, 1974*a*,*b*), electrophoretic investigations (Breuil & Guillaume, 1985; Arano & Arntzen, 1987) and cytogenetic analysis (Herrero *et al.*, 1989). Concerning morphology, inter- and intragroup variability of morphometric trait relations over the broad species range have not yet been explored. Thus, only the morphometric variability of some populations has been examined, particularly populations consisting of both paedogenetic and metamorphic individuals (Dzukic & Kalezic, 1984; Kalezic *et al.*, 1989; Kalezic *et al.*, 1990), mainly to emphasize certain character differences between the two morphs.

In this paper we describe a study of the morphological differentiation of Balkan alpine newt populations, including taxonomical implications of this differentiation. Populations of all previously recognized Balkan subspecies along with populations from the margins and from the main body of the species range were examined by standard multivariate procedures. Paedogenetic population samples were included in order to examine the influence of paedogenesis on alpine newt morphological differentiation.

Correspondence: K. Sotiropoulos, Zoological Museum, Department of Biology, University of Athens, GR 157 84 Panepistimioupolis, Athens, Greece.
E-mail: ksotirop@cc.uoa.gr

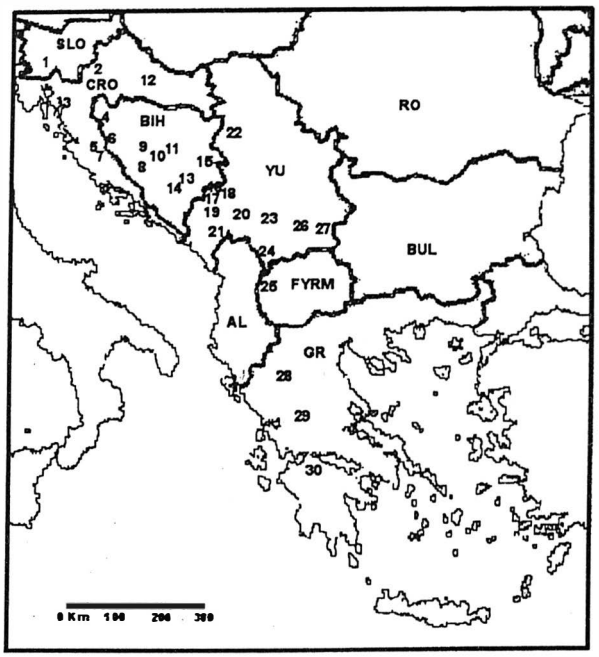


FIG. 1. Locations of the sampling sites. For locality numbers see the Appendix.

MATERIALS AND METHODS

STUDIED POPULATIONS

Locations of thirty alpine newt population samples from the Balkans are presented in Fig. 1. Locality names, their altitudes, UTM codes (10 x 10 km squares), and the numbers of males and females collected, are listed in the Appendix. Altogether, 1737 sexually mature individuals were subjected to analysis. The average numbers of males and females per sample (±SD) were 26.2±2.5 and 31.7±3.4, respectively.

Paedogenetic individuals were recognized by the presence of elongated external gills along with apparent reproductive maturity (enlarged testes in males and vitellogenic ova in females). Specimens were preserved in 70% ethanol for varying periods of time (1 to 7 years) before morphometric and qualitative characters were scored. Amphibians shrink when preserved in alcohol or formalin, especially during the first two years of preservation (Dolmen, 1983). Verrell (1985), reported a maximum body shrinkage of 3.2% in formalin-preserved smooth newts (*T. vulgaris*) during the first year of preservation. We believe that the bias introduced due to specimen preservation is evenly distributed and not systematic. The specimens analysed are deposited in the collections of G. Dzukic (Institute for Biological Research, Belgrade) and K. Sotiropoulos (Zoological Museum, University of Athens, Athens).

For each individual newt, eight external morphometric traits were measured to the nearest 0.1 mm, and fourteen qualitative characters of both sexes were scored (see Appendix for description).

DATA ANALYSIS

To determine the degree of variation in morphometric characters between (1) sexes and (2) *a priori* designated populations and intraspecific groups (i.e. presumed subspecies), a Multivariate Analysis of Variance (MANOVA) was used.

Paedogenetic populations were examined for patterns of morphometric variation, using a Principal Component Analysis (PCA) on pooled measurement data. The first component (PC1) of this analysis – which is highly positively correlated with the original data – was used as a latent size variable, while the second principal component (PC2) measures organism shape independent of size (Bookstein *et al.*, 1985 and references therein). The analysis was performed on the variance-covariance matrix of log-transformed variables (in order to meet the assumption of homoscedasticity; Zar, 1984).

Discriminant Canonical Analysis (DCA), which maximizes variation between *a priori* groups, was used to characterize the degree of divergence among populations (James & McCulloch, 1990). Discriminant canonical variates were calculated and centroids of each population were plotted on the first three canonical axes. As a measure of morphometric distance, Mahalanobis' generalized distance (D^2) was calculated between all pairs of the examined populations. This measure, which is the most popular for continuous variables, considers differences in means, variance, and covariance of characters among groups.

Variation of qualitative characters was analysed using a Correspondence Analysis following the algorithm of Greenacre (1984). Individuals received a score on each qualitative character consisting of a discrete value for the colour and/or shape trait. The output of such an analysis was the coordinates of the row (populations) and column (character states) on correspondence axes superimposed on the scatter plot.

All analyses were performed with STATISTICA 5.0 (Statsoft Inc., 1997) computer software.

RESULTS

SEX AND LOCATION EFFECTS ON MORPHOMETRIC VARIABILITY

The alpine newt displays a high degree of sexual dimorphism. Females are much larger than males (Kalezic *et al.*, 1992). The effect of sexes on the morphometric variability of the Balkan populations appeared to be highly significant, as was the effect of different localities (geographic variation). Test criteria of the MANOVA statistic (Hotelling's trace) showed a highly significant variation ($P<0.001$) between both sexes and locations, and included an interaction between these two factors. Intrapopulation paedotypic/metamorphic partitioning in terms of morphological variation was pronounced (Dzukic & Kalezic, 1984; Kalezic *et al.*, 1989; Kalezic *et al.*, 1990). Conse-

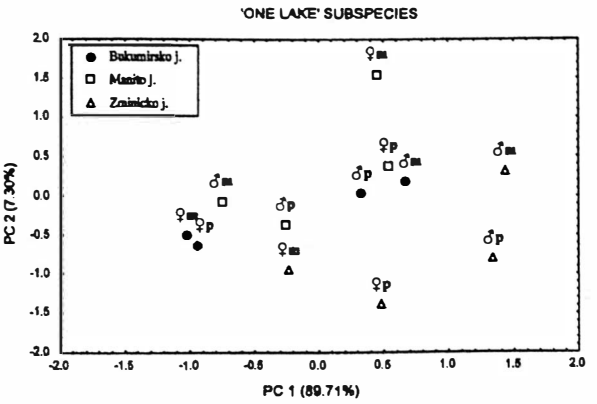


FIG. 2. Plot of the average values of PC1 and PC2 scores of paedotypic (p) and metamorphic (m) males and females of Bukumirsko, Manito and Zminicko lakes.

quently data for different sexes and different morphs (paedotypic and metamorphic) were analyzed separately.

EFFECT OF PAEDOGENESIS ON MORPHOMETRIC VARIABILITY

Three paedogenetic populations (Bukumirsko, Manito and Zminicko lakes), with sufficient numbers of individuals of each sex and morph, were analysed for body size and shape relations. Component loading patterns appeared to be the same for almost all morphometric characters across sexes and morphs. All variables were positively correlated with the first principal component (PC1), which explained more than 89.7% of the total variability and was dominated by the total body length (L). The second component contained much less variability (7.30%) and was dominated by trunk (Lsv) and tail (Lcd) lengths with contrasting signs, expressing therefore the relative tail length. The third principal component (PC3) took something more than 1% of the total variation, and therefore was not further considered.

In the morphospace delimited by the size (PC1) and shape (PC2) components, female/male and paedogenetic/metamorphic population partitioning in terms of intrapopulation group centroid positions followed the same trends (Fig. 2). More or less, the same sex of different morphs appeared to be closer to each other than the same morphs of different sexes, differentiating more on PC1 than on PC2.

INTERGROUP DIFFERENCES

The Mahalanobis' distances (D^2), calculated between all analysed population samples, appeared to be statistically significant (at least at P=0.05 level) in the majority of pairwise comparisons. Metamorphic newts of two geographically remote populations (Nos. 2 and 15, both sexes), as well as paedogenetic males of two population pairs (Nos. 6 and 17; 17 and 18), expressed non-significant D^2 values. The UPGMA phenograms (available from authors upon request), constructed on the basis of Mahalanobis' distances, clustered populations irrespectively of their presumed subspecific designation. As an exception to some degree, *T. a. veluchiensis* populations clustered together when metamorphosed females were compared. Generally, population relations did not follow their geographical proximity in the cases of either sexes or morphs.

The first three discriminant axes (DA) explained almost equal amount of the total variation in metamorphic males (74.7%) and females (75.1%), as well as in paedogenetic males (98.1%) and females (92.1%) (Table 1). The pattern of character correlation between population variability and canonical axes was considerably different especially between morphs. None of the previously described subspecies stood out as distinct, according to the discriminant analysis (Figs. 3-4). An exception, to some extent, was *T. a. veluchiensis* (Nos. 28-30). Plotting the second versus the third discriminant axis, provided clear separation of this subspecies on the ground of female affinities in comparison to other presumed intraspecific groups (Fig. 4), but with less clear separation for males (Fig. 3). The discrimination of female *veluchiensis* was

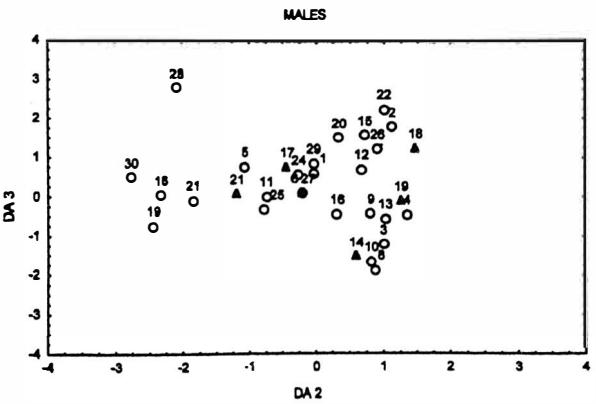


FIG. 3. Combined plot of population centroids for paedogenetic (solid triangle) and metamorphic (open circle) males on the second and third discriminant axes (DA). For population numbers see Appendix.

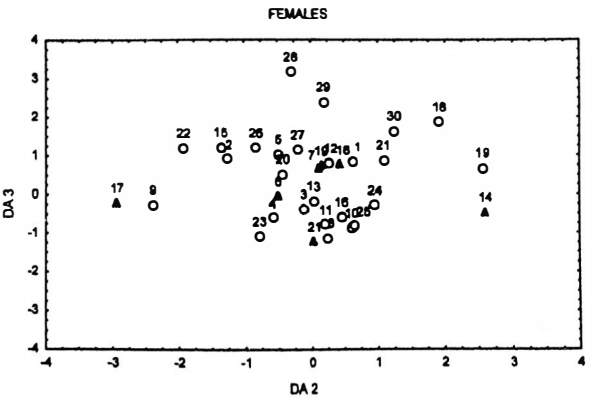


FIG. 4. Combined plot of population centroids for paedogenetic (solid triangle) and metamorphic (open circle) females on the second and third discriminant axes (DA). For population numbers see Appendix.

TABLE 1. Standardized coefficients for the first three discriminant axes (DA) of variation in morphometric characters, for metamorphic and paedogenetic females and males respectively. For character abbreviations and description, see Appendix.

METAMORPHIC

	Females			Males		
Characters	DA1	DA2	DA3	DA1	DA2	DA3
L	-0.4642	0.4326	-0.0727	-0.2847	-0.6869	-0.4333
Lsv	-0.0934	0.1048	-0.1049	-0.0997	-0.2939	0.1835
D	-0.3977	0.7049	0.2411	0.4999	-0.3545	0.8840
Lcd	0.2653	-0.2570	-0.1262	0.5974	0.1685	-0.1987
Pa	0.0562	-0.0995	0.2651	-0.1387	0.4434	-0.1142
Pp	0.4974	0.2218	0.7401	0.9102	0.4864	-0.1272
Ltc	0.3099	0.0001	-1.0776	-0.4476	-0.3854	-0.6848
Lc	0.7064	-0.1364	0.0366	-0.1607	0.3842	0.1170
Eigenvalue	1.8727	1.1836	0.9859	1.5747	1.2410	0.6501
% explained variation	34.81	56.81	75.14	33.93	60.67	74.68

PAEDOGENETIC

	Females			Males		
Characters	DA1	DA2	DA3	DA1	DA2	DA3
L	-0.5391	-0.5740	0.7215	1.2330	-0.5459	-1.6504
Lsv	0.1734	1.7015	-0.8811	0.0233	0.5743	-0.5288
D	0.0570	-0.6113	0.1448	-0.7848	-0.4127	0.3356
Lcd	0.4613	0.3052	-0.9556	-0.4118	-0.5264	1.8567
Pa	-0.2873	0.3013	0.5809	-0.0301	0.9222	0.8414
Pp	0.4241	0.2663	0.4073	0.5046	0.3768	0.0267
Ltc	-0.6806	-0.4340	-0.6420	-1.1106	-0.0446	0.1864
Lc	-0.5441	-0.5519	0.5976	-0.4939	-0.4108	-0.0124
Eigenvalue	3.0920	0.7960	0.5420	4.7847	1.2750	0.2503
% explained variation	64.28	80.83	92.10	74.40	94.23	98.12

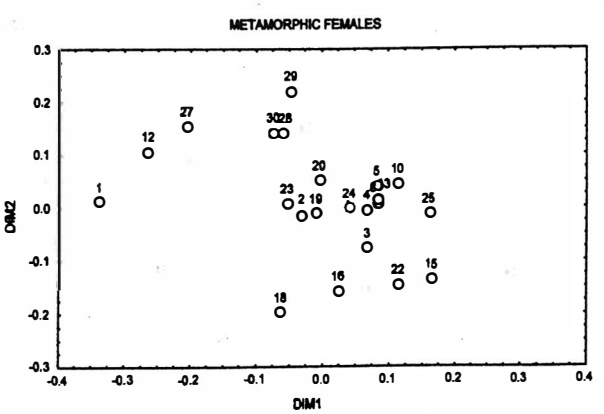


FIG. 5. Plot of populations for metamorphic females on the first and second correspondence axes (DIM). For population numbers see Appendix.

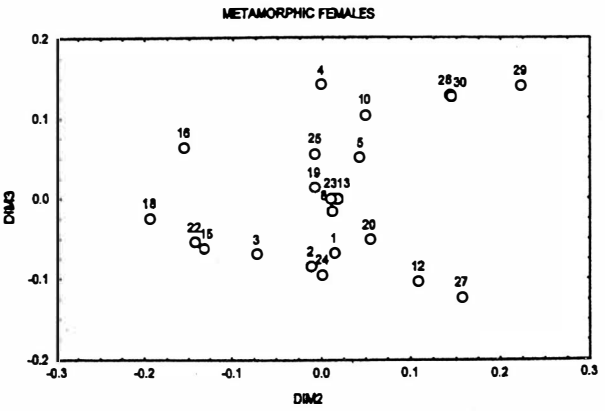


FIG. 6. Plot of populations for metamorphic females on the second and third correspondence axes (DIM). For population numbers see Appendix.

based primarily on the influence of Ltc (negative) and Pp (positive) on the third discriminant function (Table 1). Female *veluchiensis* are characterized by small Ltc value (= narrow head) and large value of Pp (= long hind limbs) in relation to values of other characters. Less clear discrimination of male *veluchiensis* was influenced by L (negative), Pa and Pp (positive) on the second discriminant function (Fig. 3, Table 1). Male *veluchiensis* exhibit a large relative body size (L) and short fore- and hind-limbs (Pa, Pp). Another obvious population grouping included the previously described 'one-lake' subspecies from Zminicko, Manito and Bukumirsko (Nos. 18, 19 and 21) (Figs. 3-4). These populations bore a considerable morphometric resemblance to each other for both sexes and compared axes pairs, especially in the case of metamorphic individuals. Metamorphic males of these populations exhibit the same pattern as *veluchiensis* males, while females are less clearly discriminated in DA2, exhibiting relatively large inter-limb distance (D) (Table 1). Apparently, the same morphs of these paedogenetic populations appeared more uniform than the alternative phenotypes of the same breeding units. Moreover, a number of spatially closer populations drifted apart, showing considerable morphometric differentiation in comparison to geographically remote populations. Populations from the eastern part of the Balkan Peninsula (Nos. 26 and 27) were well within the group of western populations (Figs. 3-4).

We did not find a great deal of variation in qualitative characters of the examined populations, especially of males in which some of the traits (IX - 1, XI - 2 and XIV - 1) appeared to be monomorphic. Correspondence analysis revealed that the males were grouped in an unrecognizable manner, without obvious taxonomic meaning. However, the females of the three *T. a. veluchiensis* populations appeared to be distinct from the other populations, and were grouped in the morphospaces delimited by DIM1/DIM2 and DIM2/DIM3 axes (Figs. 5 and 6). Females of this subspecies were mainly discernable from the females of other populations on the basis of the dominant phenotype characterized by the following set of qualitative traits: olive-greenish colour of the upper body surface and flanks; presence of black spots on the belly zone; the skin flaps of the upper jaw not reaching the lower jaw; snout from the top view narrow and convex; the flank colour pattern; and sparse dark spots on the lower edge of the tail. Discrimination of other populations did not reveal any spatially consistent pattern.

DISCUSSION

Our study of geographic patterns of morphometric and qualitative trait variability in the Balkan alpine newt does not support currently recognized intraspecific taxonomic differentiation. Two 'one-lake' subspecies, *T. a. montenegrinus* and *T. a. piperianus*, have been found to be genetically virtually indistinguishable from each other and from the nominotypical subspecies from the Balkans (Breuil & Guillaume, 1985). Electrophoretic analysis (Arano & Arntzen, 1987) and cytogenetic study (Herrero *et al.*, 1989) revealed that *T. a. reiseri*, another 'one-lake' subspecies, is genetically most similar to Serbian *T. a. alpestris*. We found no relevant morphological characters allowing for the distinction of these populations as separate taxonomic units. Our results are in agreement with Rocek's (1974*a*,*b*) observations that the degree of variability in nominotypical populations may be higher than the observed interpopulation variability of separate subspecies, making the existence of numerous Balkan subspecies questionable.

The only exception is the southernmost subspecies, *T. a. veluchiensis*, which appeared to be distinct from the other populations, especially on the grounds of qualitative morphological traits. This taxon belongs to a group of five subspecies (*T. a. cyreni*, *T. a. apuanus*, *T. a. inexpectatus*, *T. a. serdarus* and *T. a. veluchiensis*) which, according to the analyses of intraspecific genetic differentiation, may warrant full subspecific status (Breuil, 1986; Arano & Arntzen, 1987; Herrero *et al.*, 1989; Arano *et al.*, 1991). Another subspecies from this group, *T. a. serdarus*, failed to show morphological distinctiveness as a separate taxonomic unit. The population from Zminicko displays discordant patterns of morphological and genetic differentiation. Neither have clear morphological differences been identified for three Italian subspecies (*T. a. alpestris*, *T. a. apuanus* and *T. a. inexpectatus*; Giacoma *et al.*, 1988) in spite of their considerable genetic differentiation (Breuil, 1986). It seems that *T. alpestris* is an exception among highly polytypic European newts in that intraspecific taxonomic subdivision is not accompanied by substantial morphological differentiation. In contrast, the smooth newt (*T. vulgaris*) is very different, as it expresses well-defined male epigenetic characteristics for each subspecies along with a morphologically recognizable zone of intergradation (Raxworthy, 1990; Krizmanic, Mesaros, Dzukic & Kalezic, 1997). Studies of biochemical polymorphisms definitely provide the first widely applicable means of evaluating the genetic structure and continuity of populations in a manner independent of morphological variation (Larson, 1989). However, it is well established that the morphological differentiation among populations is not always closely correlated with their genetic compatibility (Cracraft, 1989).

Among European newts, the alpine newt frequently displays paedogenesis. Apart from a number of Dinaric populations (Dzukic *et al.*, 1990), paedogenesis has been reported also in *T. a. apuanus* (Andreone & Dore, 1991; Denoël, 1997; Bovero, Giacoma & Andreone, 1997), *T. a. veluchiensis* (Breuil & Parent, 1987, 1988) and *T. a. inexpectatus* (Dubois & Breuil, 1983; Andreone & Dore, 1991). As in other newt species, the incidence of *T. alpestris* paedogenesis is a pond-dependent, temporary and variable trait, as paedogenetic individuals can metamorphose, bypassing the eft stage

in their individual life cycle. The two morphs interbreed freely and there is no genetic distinctiveness, as has been revealed by electrophoresis (data for Manito; Breuil & Guillaume, 1985). As mentioned above, many Balkan subspecies have been described exclusively on the basis of morphological differences between paedogenetic and nominotypical specimens, without taking into account the within-pond morphological dimorphism. Apparently, the diagnostic features used by previous authors to describe new paedogenetic taxa were mere within-morph – or even individual – variations. Paedogenesis is generally regarded as a notorious source of morphological convergence between what are otherwise dissimilar forms, i.e. paedogenetic alpine newts of different populations are generally more similar to each other than metamorphosed adults of the same populations. This statement is valid, with a few exceptions, for Balkan paedogenetic populations. In conclusion, current evidence indicates that previously described 'one-lake' alpine newt subspecies from the Dinaric region – with the possible exception of *T. a. serdarus* – are populations that do not merit subspecific distinction.

The cause of alpine newt differentiation processes might be the high breeding site fidelity of adult newts, as has been confirmed by homing experiments (Joly & Miaud, 1989). Dispersal behaviour of the alpine newt is still unknown, but – according to studies on other newt species – a distance of 500 to 1000 m from a potential source is a reasonable dispersal estimate (see Joly & Grolet, 1996). As a result of adult newts homing to a particular aquatic site, gene flow between populations must have been impeded for a long time. Bottleneck effects and random drift in allele frequencies have been proposed as plausible factors which have facilitated genetic isolation even between geographically adjacent populations of *T. a. cyreni* in Spain (Arano *et al.*, 1991). This fact, along with species range fragmentation during Pleistocene glaciations, might have caused a substantial differentiation between subspecies. The apparent morphological similarity of the majority of Balkan alpine newt populations can be attributed to the action of stabilizing selection which remains the most commonly invoked explanation of morphological evolutionary stasis (Williamson, 1987). Stabilizing selection on an optimal phenotype leads to the adaptation of this phenotype in constant environments. However, adaptation due to uniform selection does not always prevent the genetic divergence of conspecific populations, but sometimes accelerates it (Cohan, 1984).

ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Prof. Dr. Milutin Radovanovic, the distinguished herpetologist who worked extensively on alpine newt paedogenesis, on the 30th anniversary of his death (August 1968). Our colleague, Ruza Radonjic, contributed considerably in measuring most of the former-Yugoslav populations. The technical assistance of Dragana Novakovic is highly appreciated. Dr Clive Cummins and two anonymous referees considerably improved the manuscript. Tanya Hall kindly improved the English language.

REFERENCES

Andreone, F. & Dore, B. (1991). New data on paedomorphism in Italian populations of the alpine newt, *Triturus alpestris* (Laurenti, 1768) (Caudata: Salamandridae). *Herpetozoa* **4**, 149-156.

Arano, B. & Arntzen, J. W. (1987). Genetic differentiation in the alpine newt, *Triturus alpestris*. In: *Proceedings of the 4th O.G.M. of Societas Europea Herpetologica*, 21-24. Van Gelder, J. J., Strijbosch, H. and Bergers, P. J. M. (Eds.). Nijmegen: Faculty of Sciences.

Arano, B., Arntzen, J. W., Herrero, P. & Garcia-Paris, M. (1991). Genetic differentiation among Iberian populations of the Alpine newt, *Triturus alpestris*. *Amphibia-Reptilia* **12**, 409-421.

Bookstein, F., Chernoff, B., Elder, R., Humphries, J., Smith, G. & Strauss, R. (1985). *Morphometrics in Evolutionary Biology*. Philadelphia: Academy of Natural Sciences.

Bovero, S., Giacoma, C. & Andreone, F. (1997). Sexual selection and facultative paedogenesis in the alpine newt *Triturus alpestris apuanus* (Bonaparte, 1839). Geobios, M.S. **21**, 287-292.

Breuil, M. (1986). Biologie et différentiation génétique des populations du Triton Alpestre (*Triturus alpestris*) (Amphibia, Caudata) dans le Sud-Est de la France et en Italie. Thèse du 3ème cycle, Université de Paris-Sud.

Breuil, M. & Guillaume, C-P. (1985). Étude electrophorétique de quelques populations de Tritons alpestres neoténiques (*Triturus alpestris*, Amphibia, Caudata, Salamandridae) du sud de la Yugoslavie. *Bulletin de la Société Zoologique de France* **109**, 377-389.

Breuil, M. & Parent, G. H. (1987). Essai de caractérisation du Triton alpestre hellénique *Triturus alpestris veluchiensis*. I. Historique et présentation de nouvelles données. *Alytes* **6**, 131-151.

Breuil, M. & Parent, G. H. (1988). Essai de caractérisation des populations du Triton alpestre hellénique. II. Relations entre le Triton alpestre hellénique et la sous-espèce nominative. *Alytes* **7**, 19-43.

Bruno, S. (1989). Introduction to a study of the herpetofauna of Albania. *British Herpetological Society Bulletin* **29**, 16-41.

Cohan, F. M. (1984). Can uniform selection retard random genetic divergence between isolated conspecific populations? *Evolution* **38**, 495-504.

Cracraft, J. (1989). Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In: *Speciation and its consequences*, 28-59. Otte, D. & Endler, J. A. (Eds.). Sunderland: Sinauer Associates.

Denoël, M. (1997). Un cas de néoténie, dans un bâtiment désaffecté, chez le triton alpestre, *Triturus alpestris apuanus* (Salamandridae). *Alytes* **15**, 99-103.

Dolmen, D. (1983). Growth and size of *Triturus vulgaris* and *T. cristatus* (Amphibia) in different parts of Norway. *Holarctic Ecology* **6**, 356-371.

Dubois, A. & Breuil, M. (1983). Découverte de *Triturus alpestris* (Laurenti, 1768) en Calabre (Sud de l'Italie). *Alytes* **2**, 9-18.

Dzukic, G. & Kalezic, M. L. (1984). Neoteny in the alpine newt population from the Submediterranean of Yugoslavia. *Alytes* **3**, 11-19.

Dzukic, G., Kalezic, M. L., Tvrtkovic, N. & Djorovic, A. (1990). An overview of the occurrence of paedomorphosis in Yugoslav newt (*Triturus*, Salamandridae) populations. *British Herpetological Society Bulletin* **34**, 16-22.

Ernst, F. (1952). Biometrische Untersuchungen an schweizerischen Populationen von *Triton alpestris* (Laur.). *Revue Suisse Zoologie* **59**, 399-476.

Giacoma, C., Picariello, O., Puntillo, D., Rossi, F. & Tripepi, S. (1988). The distribution and habitat of the newt (*Triturus*, Amphibia) in Calabria (Southern Italy). *Monitore zool. ital.* (N.S.) **22**, 449-464.

Greenacre, M. J. (1984). *Theory and applications of correspondence analysis.* New York: Academic Press.

Griffiths, R. A. (1996). *Newts and Salamanders of Europe.* London: T & A D Poyser.

Herrero, P., Arano, B. & Garcia de la Vega, C. (1989). Chromosome differentiation in the *Triturus alpestris* complex (Amphibia: Caudata). *Genetica* **79**, 27-35.

James, F. C. & McCulloch, C. E. (1990). Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annual Review of Ecology and Systematics* **21**, 129-166.

Joly, P. & Grolet, O. (1996). Colonization dynamics of new ponds, and the age structure of colonizing alpine newt, *Triturus alpestris*. *Acta Oecologica* **17**, 599-608.

Joly, P. & Miaud, C. (1989). Fidelity to the breeding site in the alpine newt, *Triturus alpestris*. *Behavioural Processes* **19**, 47-56.

Kalezic, M. L., Dzukic, G. & Popadic, A. (1989). Paedomorphosis in Yugoslav alpine newt (*Triturus alpestris*) populations: morphometric variability and sex ratio. *Arhiv bioloskih nauka*, Beograd **41**, 67-79.

Kalezic, M. L., Dzukic, G. & Tvrtkovic, N. (1990). Newts (*Triturus*, Salamandridae, Urodela) of the Bukovica and Ravni Kotari regions. *Spixiana* **13**, 329-338.

Kalezic, M. L., Crnobrnja, J., Djorovic, A. & Dzukic, G. (1992). Sexual size difference in *Triturus* newts: geographical variation in Yugoslav populations. *Alytes* **10**, 63-80.

Krizmanic, I., Mesaros, G., Dzukic, G. & Kalezic, M. L. (1997). Morphology of the smooth newt (*Triturus vulgaris*) in former Yugoslavia: taxonomical implications and distribution patterns. *Acta Zoologica Academiae Scientiarum Hungaricae* **43**, 345-357.

Larson, A. (1989). The relationship between speciation and morphological evolution. In: *Speciation and its consequences*, 579-598. Otte, D. & Endler, J. A. (Eds.). Sunderland: Sinauer Associates.

Raxworthy, C. J. (1990). A review of the smooth newt (*Triturus vulgaris*) subspecies, including an identification key. *Herpetological Journal* **1**, 481-492.

Reilly, S., Wiley, E. O. & Meinhardt, D. J. (1997). An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biological Journal of the Linnean Society* **60**, 119-143.

Rocek, Z. (1974*a*). Biometrical investigations of central European populations of the alpine newt, *Triturus alpestris alpestris* (Laurenti, 1768) (Amphibia: Urodela). *Acta Universitatis Carolinae Biologica* **1972**, 295-373.

Rocek, Z. (1974*b*). Beitrag zur Erkwenung der Neotenie des Alpen molches *Triturus alpestris* (Laurenti, 1768). *Vestnik Ceskoslovenske spolecnosti zoologicke* **38**, 285-294.

Sotiropoulos, K., Legakis, A. & Polymeni, R. M. (1995). A review of the knowledge on the distribution of the genus *Triturus* (Rafinesque, 1815) in Greece (Caudata: Salamandridae). *Herpetozoa* **8**, 25-34.

StatSoft, Inc. (1997). STATISTICA release 5 (Quick Reference), 3rd Edition, 1-238. Tulsa.

Verrell, P. A. (1985). Getting into a pickle with preserved specimens: formalin and distortion in the smooth newt, *Triturus vulgaris*. *Herpetological Journal* **1**, 39-40.

Williamson, P. G. (1987). Selection or constraint: a proposal on the mechanism for stasis. In: *Rates of Evolution*, 129-142. Campbell, K. S. W. & Day, M. F. (Eds.). London: Allen & Unwin.

Zar, J. H. (1984). *Biostatistical Analysis.* 2nd Edition. New Jersey: Prentice-Hall.

Zuiderwijk, A. (1997). *Triturus alpestris* (Laurenti, 1768). In: *Atlas of Amphibians and Reptiles in Europe*, 72-73. Gasc, J.-P. et al., (Eds.), Societas Europaea Herpetologica, Museum National d'Histoire Naturelle, Paris.

Accepted: 1.6.00

APPENDIX

Localities of studied populations (local spelling is given, added is the name of the nearest settlement or the mountain massif), their altitudes, location positions in the UTM grid (10 x 10 km quadrant code), date of sampling and number of males + females examined. Abbreviations: MM – metamorphic males, MF – metamorphic females, PM – paedotypic males, PF – paedotypic females. Slo - Slovenia, Cro - Croatia, BiH –Bosnia and Herzegovina, Mtg – Montenegro, Ser – Serbia, FYRM – Former Yugoslav Republic of Macedonia, Gre – Greece. 1. Ig, Kremenica (Slo, 320 m above sea level, VL68, 19 MM + 20 MF, April '81). 2. Pescenica, Velika Gorica (Cro, 105 m, WL95, 19 MM + 20 MF, April '81). 3. Kuterevo, Lika (Cro, 560m, WK16, 32MM + 43 MF, April '79). 4. Laudonov gaj, Krbavsko polje (Cro, 630 m, WK44/54, 26 MM + 28MF, April '89). 5. Usljebrka, Zegar, (Cro, 80 m, WJ68, 29 MM + 34 MF, May, '85). 6. Pajica lokva, Macure (Cro, 275 m, WJ78, 26 PM + 49 PF, May '85). 7. Grulovici, Kistanje (Cro, 245 m, WJ77, 0 PM + 36 PF, May '85). 8. Karaizovci, Glamocko polje (BiH, 890 m, XJ56, 38MM + 43 MF, May '83). 9. Rasticevsko jezero, Blagaj (BiH, 1180 m, XJ77, 46MM + 30MF, June '85). 10. Supljica, Kupreska vrata (BiH, 1350 m, XJ87, 27MM + 48MF, May '83). 11. Prokosko jezero, Mt. Vranica (BiH, 1636 m, YJ27, 50 MM + 50MF, July '80). 12. Jankovac, Mt. Papuk (Cro, 457 m, YL04/14, 19 MM + 20 MF, May '81). 13. Gornje bare, Mt. Zelengora (BiH, 1650 m, CP00, 40MM + 26 MF, May '83). 14. Sopilji, Nevesinjsko polje (BiH, 850 m, BP60, 9 PM + 19 PF, May '83). 15. Seljani, Rogatica (BiH, 820 m, CP45, 20 MM + 20 MF, April '91). 16. Zminje jezero, Mt. Durmitor (Mtg, 1495 m, CN48, 45 MM + 58 MF, July '79). 17. Vrazje jezero, Mt. Durmitor (Mtg, 1411 m, CN47, 2PM + 16 PF, July '79). 18. Zminicko jezero, Mt. Sinjavina (Mtg, 1285 m, CN57, 19 MM + 10 MF + 9 PM + 18 PF, July '79, August '81). 19. Manito jezero, Lukavica (Mtg, 1773 m, CN54, 50 MM + 48 MF + 36 PM + 50 PF, July '86). 20. Ursulovacko jezero, Mt. Bjelasica (Mtg, 1760 m, CN94, 12 MM + 27 MF, July '97). 21. Bukumirsko jezero (Mtg, 1440 m, CN71, 19 MM + 14 MF+ 49 PM + 49 PF, June '84). 22. Joseva, Valjevo (Ser, 345 m, DQ00/01, 20 MM + 20 MF, April '98). 23. Savine vode, Mt. Mokra Gora (Ser, 1680 m, DN64, 0MM + 26 MF, June '77). 24. Donje ravne mlake, Mt. Sara (Ser, 2100 m, DM74, 18 MM + 20 MF, July '95). 25. Podgorecko jezero, Mt. Jablanica (FYRM, 1870 m, DL66, 19 MM + 21 MF, September '96). 26. Mt. Sveti Ilija, Vranje (Ser, 1120 m, EN61, 45 MM + 36 MF, May '80). 27. Stojkovica mahala, Vlasinsko jezero (Ser, 1340 m, FN12/13, 20 MM + 20 MF, May '81). 28. Limni Pigon Aoou (Gre, 1400 m, EK 11, 7 MM + 13MF, June '98). 29. Velouchi (=Tymphristos Mt) (Gre, 1850 m, EJ 61, 10MM + 11MF, July '95). 30. Panachaiko Mt., Rakita plateau (Gre, 1050 m, EH82, 7MM + 7MF, April-May '97).

Morphometric characters. L – total length, Lsv-snout-vent length (from the snout to the posterior edge of the cloacal base), Lcd – tail length (from the anterior edge of the cloacal base to the tail tip), Ltc – head width, Lc – head length (from the snout to the corner of the mouth), Pa – fore-limb length, Pp – hind-limb length, D – distance between fore- and hind-limb. The length of the tail in damaged individuals was estimated as the expected value from the regression of Lcd on Lsv.

Qualitative characters. I. Colour of the upper body surface and flanks – (1) dark, (2) olive-greenish, (3) brownish, (4) dove-blue, (5) whitish-grey. (6) other colour; II. Belly colour – (1) deep yellow to bright orange, (2) dark-red or reddish, (3) other colour; III. Throat colour pattern – (1) unspotted, (2) sparse black spots and/or blotches dispersed all over the throat, (3) numerous spots and/or blotches dispersed all over the throat, (4) spots and/or blotches concentrated close to gular fold; IV. Black spots and/or blotches on the belly zone – (1) present, (2) absent; V. Skin flaps of the upper jaw – (1) extend over the lower jaw; (2) do not reach lower jaw; VI. Snout from the dorsal view – (1) wide and flat, (2) narrow and convex, (3) wide and concave; VII. Flank colour pattern (scored in females only) – (1) reticulate, (2) numerous denticulate blotches present, (3) other ornamentation; VIII. Dorsal crest (scored in males only) – (1) high (>2 mm), (2) medium (1-2 mm), (3) low (<1 mm); IX. Colour of the dorsal crest (scored in males only) – (1) dark zones completely separated by narrow lightly coloured stripes, (2) above the dark zones a wavy white band underlines a row of well separated dark spots, (3) other colour pattern; X. Number of dark spots in the lower edge of tail - (1) numerous (>10), (2) moderate (3-10), (3) sparse (<3); XI. Size of dark blotches on the flanks – (1) regularly decrease toward the forelimbs, (2) no regularity; XII. The flank zone with dark blotches is – (1) equally wide along the trunk, (2) wide close to hindlimbs, and narrow close to forelimbs; XIII. Cloaca colour pattern (scored in males only) - (1) with spots, (2) with blotches, (3) completely black; XIV. Dorsal crest origin (scored in males only) – (1) on the rear part of the head, (2) between forelimbs.

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 9-15 (2001)

PREDATOR-INDUCED BEHAVIOURAL RESPONSES: TADPOLES OF THE NEOTROPICAL FROG *PHYLLOMEDUSA TARSIUS* DO NOT RESPOND TO ALL PREDATORS

BENEDIKT R. SCHMIDT[1] AND ADOLFO AMÉZQUITA[2]

[1]*Zoologisches Institut, University of Basel, Rheinsprung 9, CH-4051 Basel, Switzerland*

[2]*Depto. de Ciencias Biológicas, Universidad de los Andes, A. A. 4976, Bogotá, Colombia*

Many species show behavioural responses to predators that reduce predation mortality but are assumed to be costly. We tested whether an induced behavioural response is predator-specific and whether the strength is related to the risk of being killed by a predator. We used tadpoles of the neotropical frog *Phyllomedusa tarsius* as prey, and larvae of an aeshnid dragonfly and belostomatid bugs as predators. Belostomatids killed twice as many tadpoles within 24 hours as aeshnids did. Tadpoles reduced activity in the presence of aeshnids by 30% but did not respond at all to the more dangerous belostomatids. Tadpoles did not show spatial avoidance of predators. We favour the explanation that tadpoles of *P. tarsius* did not respond to belostomatids because belostomatids are encountered too rarely for evolution to favour an induced response to belostomatids.

Key words: amphibian, distribution, induced response, *Phyllomedusa tarsius*, predation risk, tadpole

INTRODUCTION

Predators are well-known for inducing antipredator responses in their prey (Tollrian & Harvell, 1999). The responses include changes in life history (Skelly & Werner, 1990; Sih & Moore, 1993; Warkentin, 1995), morphology (Smith & Van Buskirk, 1995; Van Buskirk, McCollum & Werner, 1997, Van Buskirk & Schmidt 2000), and behaviour (Lawler, 1989; Horat & Semlitsch, 1994; Anholt & Werner, 1995). These changes in life history, morphology or behaviour can reduce predation rates but the induced phenotype generally suffers a cost, usually reduced growth rates (e.g., Skelly, 1992; Skelly & Werner, 1990; Van Buskirk et al., 1997; Relyea & Werner, 1999; Van Buskirk 2000).

Because there is a cost to an antipredator response, natural selection should favour precise antipredator responses. The cost of ignoring a dangerous predator is an increased probability of death, whereas the cost of overestimating risk is a loss of opportunities to feed or mate. Therefore, an induced response should be related to the risk of predation and be predator-specific where predators differ in predation risk (Sih, 1987). This prediction has been tested several times with one predator and two prey species. These studies generally showed that the more vulnerable prey species reacted more strongly (see Sih, 1987 for a review). Such studies are at risk of confounding predation risk and interspecific differences between prey species. Several studies have measured behavioural responses of one prey species to different predators, but only a few have related behaviour to predation risk (Skelly, 1994; Anholt & Werner, 1995; Lefcort, 1996; Van Buskirk & McCollum 2000). This study aims at testing whether one species of prey reacts differently to two species of predator that differ in predation threat.

Tadpoles offer an excellent opportunity to test whether an induced response is related to the risk of predation. They show predator-induced responses in behaviour, morphology, life history or habitat use (Lawler, 1989; Stauffer & Semlitsch, 1993; Warkentin, 1995; Smith & Van Buskirk, 1995). Behavioural responses often include changes in levels of activity (Lawler, 1989; Skelly & Werner, 1990; Horat & Semlitsch, 1994). More active individuals (or species) have a higher probability of being captured by a predator (Cooke, 1971; Woodward, 1983; Azevedo-Ramos, Van Sluys, Hero & Magnusson, 1992; Skelly, 1994; Lefcort, 1996; Van Buskirk & McCollum 2000). Activity level is also related to feeding and growth rates with more active individuals feeding more and growing faster (Skelly & Werner, 1990; Werner, 1991; Skelly, 1992; Relyea & Werner, 1999). Therefore, tadpoles have to trade-off growth and mortality rates that both depend on behaviour.

In this study, we experimentally tested whether tadpoles of the neotropical frog *Phyllomedusa tarsius* (Anura: Hylidae) reduce activity and spatial distribution in the presence of two predator species. We tested whether the reduction in activity and the change in spatial distribution are predator-specific, and whether the strength of the induced response is related to the risk of predation.

Correspondence: B. Schmidt, Zoologisches Institut, University of Zurich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. *E-mail*: bschmidt@zool.unizh.ch

MATERIAL AND METHODS

STUDY SITE AND STUDY ANIMALS

The study was carried out in reserve 1501 of the Biological Dynamics of Forest Fragments Project (Instituto Nacional de Pesquisas da Amazônia/Smithsonian Institution), 80 km north of Manaus, Brazil (Gascon, 1991, 1992; Zimmerman & Simberloff, 1996). We used a small area of *terra firme* forest cleared of understory to conduct the experiments under natural light conditions. We chose tadpoles of *Phyllomedusa tarsius* as prey because Azevedo-Ramos *et al.* (1992) showed that less active tadpoles of this species get killed less often by aeshnid dragonfly larvae. We chose larvae of an aeshnid dragonfly and adult belostomatid giant water bugs as predators because (1) given the size difference, they were likely to differ with respect to predation risk; (2) we had previous information about their effects on tadpole communities (Gascon, 1992); (3) both are sit-and-wait predators (Kehr & Schnack, 1991; Pritchard, 1965); (4) both use visual (and tactile) cues for prey detection (Peckarsky, 1984; most invertebrate predators detect moving prey more easily than stationary prey [Wellborn, Skelly & Werner, 1996]); and (5) tadpoles of other species are known to change behaviour in response to both predators (Kiesecker, Chivers & Blaustein, 1996).

Tadpoles and predators were collected from shallow ponds (see Gascon, 1991) a few days prior to the experiment. There were no aquatic plants in the source ponds. Tadpoles were kept together in a large plastic tub prior to the experiments. Tadpoles occurred with both predators. Thus, our tadpoles were unlikely to be predator-naive (e.g. Chivers, Wisenden & Smith, 1996). We found no belostomatids in ponds where we collected aeshnids and vice versa, but they often do coexist (Gascon, 1992; Hero, Gascon & Magnusson, 1998). We formed groups of tadpoles from the stock for our experiments. Thus, each group contained some tadpoles that experienced aeshnids and some that experienced belostomatids.

The ponds sampled contained tadpoles of other anurans (*Phyllomedusa tomopterna*, *Hyla minuta*, *Osteocephalus taurinus* and *Bufo marinus*), and various other predators (hemipterans, spiders, and dragonfly and damselfly larvae).

Tadpole snout-vent length was on average 9.6 mm (range: 5.0 to 12.8 mm). Gosner (1960) developmental stages were between 25 and 30. This sample represents the range of sizes and developmental stages of *P. tarsius* tadpoles we encountered in the field in August 1995. Belostomatids had a mass of 6.0±0.97 g (mean±SD; n=10) and a length 64.1±3.95 mm. The mass of an aeshnid was 0.5±0.27 g and length was 32.6±6.43 mm (n=9). Belostomatids were adults whereas aeshnids were at mid- to late-developmental stages. All predators were able to kill all sizes of tadpoles.

EXPERIMENTS

We measured behavioural responses of tadpoles to predators and predation risk in separate experiments during August 1995. We used circular plastic basins (diameter 30 cm, depth 11 cm; Gascon, 1992) filled to a depth of 5 cm with water from the same nearby stream for both experiments (*P. tarsius* never occurs in streams or streamside pools; these have very different, fish-dominated predator communities [Gascon, 1991, 1992; Zimmerman & Simberloff, 1996; Hero *et al.*, 1998]). After every trial, basins were cleaned and refilled. There was no leaf litter, nor aquatic plants in the basins (habitat complexity does not affect the difference in predation rates between aeshnids and belostomatids [Babbitt & Jordan, 1996]). We only added a small piece of twig as a perch for aeshnids.

For measuring the behavioural responses of tadpoles we added two predator cages on opposite sides of the basins. Cages were made of plastic mesh and were large enough for predators to move – tadpoles could therefore detect chemical, visual and tactile cues from predators. We then added one predator to one of the cages (or both cages were left empty for controls) and added ten tadpoles to the basins (tadpoles of *P. tarsius* may form schools [Duellman & Trueb, 1994]). We let the predators and tadpoles acclimatize to the experimental conditions for at least six hours before we started to measure behaviour. We scored each tadpole as being inactive (i.e. no visible movement) or active (i.e. tadpoles either remaining perpendicular in the water column by means of tail movements or swimming [Azevedo-Ramos *et al.*, 1992]; tadpoles are midwater filter feeders [Duellman & Trueb, 1994]). We measured activity of tadpoles seven times at intervals of five minutes. From these data we calculated the proportion of tadpoles active over the course of 30 mins. We also counted in the same way the number of tadpoles that were on the side of the basin opposite to the cage that contained the predator. We used this as our measure of spatial avoidance. We used six groups of ten tadpoles to measure behavioural responses. Groups of ten tadpoles were formed once and were chosen haphazardly from the stock of available tadpoles. Each group was tested with and without each type of predator. We only measured one treatment per group on any one day. Tadpoles were therefore tested over three days. On each day, two groups were subjected to aeshnids, belostomatids or empty predator cages. Groups were rotated through the treatments according to a latin square design.

To measure predation risk we added ten tadpoles and one predator – either belostomatid or aeshnid – to a basin at 0700 hr the first day and at 0800 hr the following day. We then counted the number of tadpoles alive every hour for a period of 16 hr, and again 24 hr after the beginning of the experiment. Predators and tadpoles were used only once. We conducted 12 replicate predation trials for each predator species over a period of three days (three to five trials per predator species and

day). Some trials were excluded from the analysis because belostomatids escaped (n=3) or aeshnids moulted (n=2).

Clearly, we measured predation risk in an environment much simpler than a natural pond. Therefore, our estimates of risk cannot directly be related to conditions in the field. However, our experimental approach does permit measurement of differences between predators without the confounding effects of predator satiation, prey density, prey size or presence of other predator species. Our experiments therefore permit analysis of the qualitative relationship between predation risk (i.e. which predator is more of a threat) and the tadpole behaviour that we are interested in. If the simple approach of using consumption rate as a measure of predation risk fails, it means that other factors may be important. We will discuss which other factors we believe are important below.

Tadpoles were fed *ad libitum* prior to the behaviour and predation experiments, but could not feed during the experiment. Prior to the behaviour measurements and predation trials, predators were fed tadpoles of both *P. tarsius* and *P. tomopterna*, with *P. tarsius* being more abundant in our samples, but were not allowed to feed for 24 hr prior to their use in the experiments.

Response Variables and Statistical Analysis

Using the SAS procedure GLM, we tested for effects of predator identity and group on the activity (proportion of tadpoles active), and spatial avoidance (proportion of tadpoles away from the predator) of tadpoles in an ANOVA after arcsine squareroot transformation of the data. We used the predator x group interaction as our error term for the construction of F-tests because groups were not replicated (Potvin, 1993). This way of analysing our data assumes the absence of a predator x group interaction. This assumption seems valid, as groups of tadpoles were assembled haphazardly. Group may be considered a random effect, but the computation of the sums of squares is the same in this analysis (Potvin, 1993). Differences among treatments were tested using Tukey's studentized range test. The critical value of the Tukey test depends on the error df of the ANOVA (Zar, 1999: 211). Thus, we used the predator x group interaction df for the computation of the Tukey test. We tested the effectiveness of the two predators (= number of tadpoles killed within 24 hr) using a Mann-Whitney U test (i. e. a Wilcoxon two-sample test in the terminology of SAS procedure NPAR1WAY). Only one belostomatid killed all ten tadpoles. Therefore data are unlikely to be censored.

RESULTS

Belostomatids killed and consumed more than twice as many tadpoles within 24 hr as did aeshnid dragonfly larvae (belostomatids: 7.6±0.57 tadpoles killed [mean±SD]; aeshnid: 3.3±0.30 tadpoles killed; Wilcoxon two-sample test, Z=3.47, P=0.0005; Fig. 1). As belostomatids were much larger than aeshnids, one might expect this result. Our intention was to test for a relationship between predation risk and the strength of behavioural responses. We therefore needed an estimate of predation risk. Body size *per se* is not an indicator of predation risk.

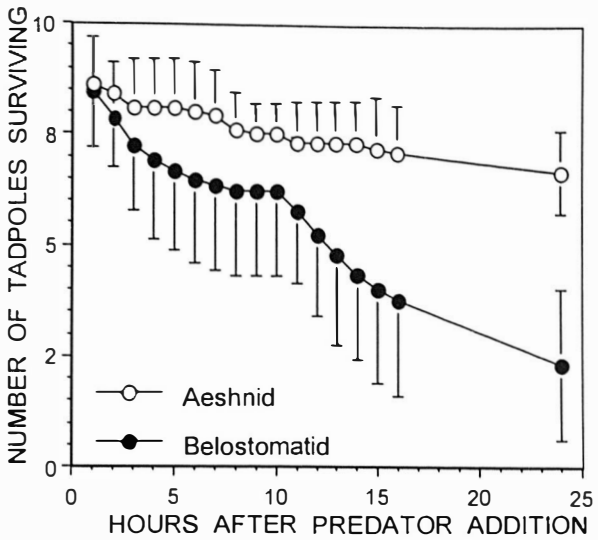


FIG. 1. Survivorship curves for tadpoles in the predation experiment. Open symbols represent predation by dragonfly larvae, filled symbols predation by belostomatids. Means± SD are from n=10 (aeshnids) and n=9 (belostomatids) replicates.

Tadpoles of *P. tarsius* where active for 49±4% of the time when no predator was present. They reduced activity significantly (biologically and statistically) in the presence of aeshnid dragonfly larvae by one third (33±4% active), but did not reduce it in the presence of belostomatids (48±4% active; Table 1, Fig. 2). Tukey's studentized range test (α=0.05) indicated no difference between the response of tadpoles in the control and belostomatid treatment, but activity of tadpoles in the presence of aeshnids was found to be different from that under both other treatments. Groups of tadpoles did not differ significantly in activity (Table 1). This suggests

TABLE 1. Summary of the univariate analyses of variance for activity level (proportion of tadpoles active or showing swimming and tail movement) and microhabitat use (proportion of tadpoles away from predator). The predator x group interaction was used as the error term. Data were arcsine square-root transformed for statistical analysis.

Source of variation	df	Mean squares	F	P
Activity				
Predator	2	0.0516	8.32	0.0075
Group	5	0.0180	2.92	0.0704
Predator x group	10	0.0062		
Spatial Avoidance				
Predator	2	0.0025	0.13	0.8804
Group	5	0.0121	0.62	0.6913
Predator x group	10	0.0197		

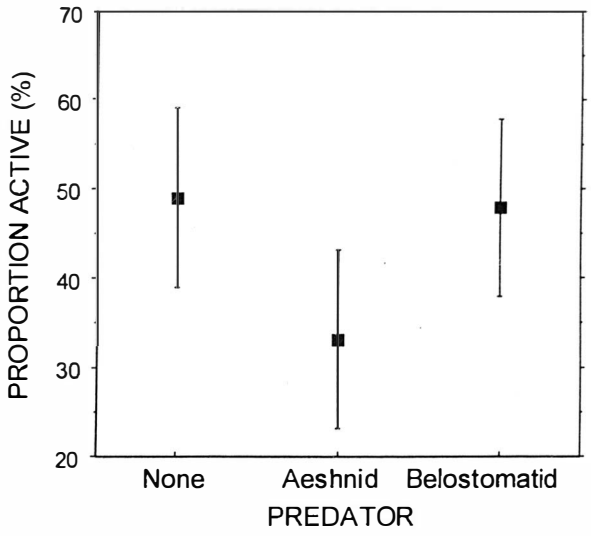


FIG. 2. Effects of predators on activity of tadpoles of *Phyllomedusa tarsius*. Means±SD are from six replicates each.

that the order of behavioural measurements did not affect behavioural responses.

Tadpoles of *P. tarsius* did not show spatial avoidance of predators (proportion away from predator: control 59±6%, belostomatid 63±4%, aeshnid 64±6%; Table 1). The Tukey test indicated no statistical difference between treatments. Groups of tadpoles did not differ significantly (Table 1).

DISCUSSION

Behavioural responses of tadpoles of *Phyllomedusa tarsius* to predatory insects were predator-specific. Tadpoles reacted strongly to the presence of aeshnid dragonfly larvae but showed no response to belostomatids. Responses to predators can be specific because tadpoles recognize different species and can discriminate between predators, or because different predators release different amounts of chemical cue and tadpoles respond to the amount of chemical cue available. We suggest that tadpoles of *P. tarsius* can discriminate between predator species. In our behaviour experiment, no cues associated with feeding were available. Only cues released by a predator after a day of starving were available (e.g. metabolic products). Because belostomatids have a mass twelve times greater than that of aeshnids, we would expect that the behavioural response to belostomatids would be stronger if it is based on the amount of cue released. This was not the case. We conclude that the behavioural responses are based on unique cues released by predators and that tadpoles use these cues for predator recognition.

The lack of behavioural response to belostomatids is unexpected for two reasons: (1) it is one of the few studies where prey did not react behaviourally to a predator that is potentially dangerous (for similar results see Sih, 1992; McPeek, 1990; Griffiths *et al.*, 1998); and (2) the response did not depend on predation risk. We first discuss why behavioural responses are not related to predation risk, and go on to discuss why tadpoles of *P. tarsius* did not respond to belostomatids at all.

The tadpoles' lack of response to an apparently high-risk predator was unexpected, but it is possible that the relative danger posed by the two predators in the field was altered in our experimental conditions. Predation rates may be lower for belostomatids in a more natural predation trial and in the field. Indeed, the impact of belostomatids on tadpole communities seems to be lower than our results suggest (most likely because they are rather rare; Hero *et al.*, 1998). Under experimental conditions, however, belostomatids appear to pose more of a threat than aeshnids. Babbitt & Jordan (1996) found in an experiment very similar to ours that juvenile *Belostoma fluminea* (average mass 0.27 g) consumed significantly more tadpoles than larvae of the dragonfly *Anax junius* (average mass 1.36 g) in predation trials (54% and 30% of all tadpoles, respectively, were consumed). Even if we overestimated predation risk, we are confident that belostomatids are potentially dangerous predators. Consequently, a behavioural response would seem beneficial. The toad tadpoles studied by Kiesecker *et al.* (1996) reduced activity in the presence of belostomatids. This suggests that reducing activity in the presence of belostomatids is adaptive because it is likely to reduce the probability of being killed by a belostomatid. Thus, based on potential killing rates and the studies by Babbitt & Jordan (1996) and Kiesecker *et al.* (1996), we expect at least a weak behavioural response. If, as we did, we find no behavioural response at all to a potentially dangerous predator then we must ask either why tadpoles do not change behaviour and which factors are responsible for the lack of a response, or how does a tadpole measure predation risk?

TABLE 2. Distribution and overlap of *Phyllomedusa* tadpoles and their predators at the study site. n=29 ponds were surveyed. Data were extracted from Hero *et al.* (1998). Absolute values are given in parentheses.

Predators	Proportion of ponds in which both *P. tarsius* and predators were found at least once	Proportion of visits in which a predator is encountered, given that it uses the pond (= proportion of time a pond is used)	Proportion of time a predator is present when *P. tarsius* tadpoles are present in a pond
Aeshnids	1.0 (14/14)	0.5	0.5
Belostomatids	0.42 (6/14)	0.25	0.105

Why do tadpoles of *P. tarsius* not respond at all to a potentially dangerous predator? A variety of factors has been proposed and shown to affect larval amphibian antipredator behaviour, and activity in particular; e.g. resource availability (e.g. Anholt & Werner 1995); predator-naïvety, experience and learning (e.g. Sih & Kats, 1994); predator diet (e.g. Laurila, Kujasalo & Ranta, 1997); presence of constitutive defences such as skin toxins (e.g., Kiesecker *et al*., 1996); predator speed (Werner & Anholt, 1993) and cues available for predator detection (e.g., Stauffer & Semlitsch, 1993). These factors can affect the strength of a response in an experiment, including the lack of response to a predator. However, they cannot explain the differential behavioural responses that we found. Conditions were the same, and both predator species and tadpoles were collected from ponds where they experienced the same environment previous to the experiment. Similarly, other factors such as feeding style (chewing versus sucking) cannot be responsible because predators could not feed while we were measuring behaviour. We therefore suggest that these factors and the experimental design are not responsible for the results we obtained.

We propose that tadpoles of *P. tarsius* do not respond to belostomatids because they encounter them too rarely. As a consequence, there was no opportunity for natural selection to shape induced behavioural responses. The reasoning is as follows. On the one hand, if organisms always encounter predators then we expect constitutive defences rather than induced defences to evolve. For example, tadpoles of the frog *Pseudacris crucifer* nearly always encounter predators during their larval life. As expected, their antipredator phenotype is such that it confers high fitness in the presence of predators (Skelly, 1995; Smith & Van Buskirk, 1995) but shows weak induced responses (Smith & Van Buskirk, 1995). On the other hand, if organisms never encounter predators then predator-induced responses do not evolve (e.g. Sih, 1986; McPeek, 1990; Parejko & Dodson, 1991; Neill, 1992; Pijanowska, Weider & Lampert, 1993). Salamander larvae that do not have contact with fish do not respond behaviourally to them (Kats, Petranka & Sih, 1988; Sih, 1992). Tadpoles of the frogs *Ascaphus truei* and *Alytes muletensis* respond to predators with which they coexist but do not respond to predators they do not usually encounter (Feminella & Hawkins, 1994; Griffiths *et al*., 1998). Induced responses will only evolve in a heterogeneous environment: prey must encounter predators sometimes, but predators must be neither ubiquitous nor absent (Via & Lande, 1985; van Tienderen, 1991; De Jong, 1995). There is likely to be an encounter rate > 0 and < 1, below and above which constitutive absence or presence will be favoured over induced defences despite environmental heterogeneity (e.g. Riessen, 1992). Adaptations should be more precise in common or source environments than in rare or sink environments (Kawecki & Stearns, 1993).

We suggest that although *P. tarsius* does encounter belostomatids from time to time, this happens too rarely for an induced response to evolve. In contrast, aeshnid dragonflies are encountered more often and an induced response has evolved. At our study site, aeshnids occur in twice as many ponds as belostomatids (Table 2; the sources of the distributional data that includes a description of the sampling methods are Gascon, 1992 and personal communication; Hero *et al*., 1998). Aeshnids are found in all ponds that are used by *P. tarsius* whereas belostomatids are found in only about 40% of the ponds. The overlap between frogs and predators in not 1.0 and 0.4, however. Aeshnids and belostomatids are found only in c. 50% and 25% of the visits to a pond, respectively (Hero *et al*., 1998; C. Gascon, personal communication). This means aeshnids are using c. 50% of the ponds at a given time whereas belostomatids use only c. 10% of the ponds at a given time (see Table 2). *Phyllomedusa tarsius* will thus only encounter belostomatids in 10% of the ponds it uses for reproduction. Even though the theory of induced responses does not give lower limits for encounter rates below which induced responses do not evolve, the spatiotemporal overlap between belostomatids and *P. tarsius* seems to have been too low for an induced behavioural response to evolve.

However, many evolutionary ecologists would argue that an overlap of 10% between predators and prey is large enough for the evolution of an induced defense (J. Van Buskirk and R. Altwegg, personal communications). Clearly, tadpoles will encounter belostomatids from time to time and there may be selection for an induced defence. The net effect of selection may be weak, however. As expected from the general positive relationship between distribution and abundance, belostomatids are not only less widely distributed than aeshnids, but they are also less abundant within ponds (Hero *et al*., 1998). Thus, they may have a low selective impact on tadpole populations even when present. Alternatively, belostomatids may have strong effects on tadpole populations (as suggested by Fig. 1). If so, the ponds with belostomatids may represent sinks (Pulliam, 1988). Few frogs will metamorphose from such ponds and most adults will have grown up in belostomatid-free ponds. As a consequence, the effect of selection may be overridden by gene flow from belostomatid-free (source) ponds (Holt & Gaines, 1992; Kawecki & Stearns, 1993; Kawecki, 1995; Holt, 1996; Storfer & Sih, 1998). In sum, selection for an induced defence will be in place from time to time, but it is likely to be weak or overridden by the effects of gene flow.

Our hypothesis for the absence of an induced behavioural defence seems plausible but we have no experimental test. However, such a test would be possible – there are other species of *Phyllomedusa* on our study site that are either more and less abundant than *P. tarsius*. In the nearby reserve of 'Adolfo Ducke' belostomatids do not occur at all. Such variation in

abundance and overlap of predators and prey could be used in undertaking a comparative test of our hypothesis.

Our expectation that behavioural responses are predator-specific was met, although in an unexpected way. Rather than showing that the induced response depends on predation risk, we showed that tadpoles of *P. tarsius* do not respond at all to a potentially dangerous predator. The predator appears to be too rare for the evolution of an induced defense. We suggest that the presence and strength of an induced defence does not only depend on predation risk but also on the abundance and distribution in space and time of predators and prey. This calls for theoretical studies that investigate how frequently prey must encounter specific predators if predator-specific induced responses are to evolve, and how abundance and predation rate interact in shaping induced responses.

ACKNOWLEDGEMENTS

We thank O. de Souza Pereira for help with field work and C. Gascon, B. Anholt, J. Van Buskirk and R. Altwegg for comments on the manuscript. The field work was done while we were participants in the field course "Ecologia da floresta amazônica" organized by the Instituto Nacional de Pesquisas da Amazônia, the Smithsonian Institution, Universidade Estadual de Campinas, and OTS and funded by USAID-BRAZIL and the MacArthur Foundation. A. Amézquita thanks specially the 'moon bath team' for support in the field. This is publication No. 324 of the Biological Dynamics of Forest Fragments Project technical series.

REFERENCES

Anholt, B. R. & Werner, E. E. (1995). Interaction between food availability and predation mortality mediated by adaptive behavior. *Ecology* **76**, 2230-2234.

Azevedo-Ramos, C., Van Sluys, M., Hero, J.-M. & Magnusson, W. E. (1992). Influence of tadpole movement on predation by odonate naiads. *J. Herp.* **26**, 335-338.

Babbitt, K. J. & Jordan, F. (1996). Predation on *Bufo terrestris* tadpoles: effects of cover and predator identity. *Copeia* **1996**, 485-488.

Chivers, D. P., Wisenden, B. D. & Smith, R. J. F. (1996). Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Anim. Behav.* **52**, 315-320.

Cooke, A. S. (1971). Selective predation by newts on frog tadpoles treated with DDT. *Nature* **229**, 275-276.

De Jong, G. (1995). Phenotypic plasticity as a product of selection in a variable environment. *Amer. Nat.* **145**, 493-512.

Duellman, W. E. & Trueb, L. (1994). *Biology of amphibians*. Baltimore: Johns Hopkins University Press.

Feminella, J. W. & Hawkins, C. P. (1994). Tailed frog tadpoles differentially alter their feeding behavior in response to non-visual cues from four predators. *J. North Amer. Benth. Soc.* **13**, 310-320.

Gascon, C. (1991). Population- and community-level analyses of species occurrences of central Amazonian rainforest tadpoles. *Ecology* **72**, 1731-1746.

Gascon, C. (1992). Aquatic predators and tadpole prey in central Amazonia: field data and experimental manipulations. *Ecology* **73**, 971-980.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

Griffiths, R. A., Schley, L., Sharp, P. E., Dennis, J. L. & Román, A. (1998). Behavioural responses of Mallorcan midwife toad tadpoles to natural and unnatural snake predators. *Anim. Behav.* **55**, 207-214.

Hero, J.-M., Gascon, C. & Magnusson, W. E. (1998). Direct and indirect effects of predation on tadpole community structure in the Amazon rainforest. *Aust. J. Ecol.* **23**, 474-482.

Holt, R. D. (1996). Demographic constraints in evolution: towards unifying the evolutionary theories of senescence and niche conservatism. *Evol. Ecol.* **10**, 1-11.

Holt, R. D. & Gaines, M. S. (1992). Analysis of adaptation in heterogeneous landscapes – implications for the evolution of fundamental niches. *Evol. Ecol.* **6**, 433-447.

Horat, P. & Semlitsch, R. D. (1994). Effects of predation risk and hunger on the behavior of two species of tadpoles. *Behav. Ecol. Sociobiol.* **34**, 393-401.

Kats, L. B., Petranka, J. W. & Sih, A. (1988). Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* **69**, 1865-1870.

Kawecki, T. J. (1995). Demography of source-sink populations and the evolution of ecological niches. *Evol. Ecol.* **9**, 38-44.

Kawecki, T. J. & Stearns, S. C. (1993). The evolution of life histories in spatially heterogeneous environments: optimal reaction norms revisited. *Evol. Ecol.* 7, 155-174.

Kehr, A. I. & Schnack, J. A. (1991). Predator-prey relationship between giant water bugs (*Belostoma oxyurum*) and larval anurans (*Bufo arenarum*). *Alytes* **9**, 61-69.

Kiesecker, J. M., Chivers, D. P. & Blaustein, A. R. (1996). The use of chemical cues in predator recognition by western toad tadpoles. *Anim. Behav.* **52**, 1237-1245.

Laurila, A., Kujasalo, J. & Ranta, E. (1997). Different antipredator behaviour in two anuran tadpoles: effects of predator diet. *Behav. Ecol. Sociobiol.* **40**, 329-336.

Lawler, S. P. (1989). Behavioral responses to predators and predation risk in four species of larval anurans. *Anim. Behav.* **38**, 1039-1047.

Lefcort, H. (1996). Adaptive, chemically mediated fright response in tadpoles of the southern leopard frog, *Rana utricularia*. *Copeia* **1996**, 455-459.

McPeek, M. A. (1990). Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. *Ecology* **71**, 1714-1726.

Neill, W. E. (1992). Population variation in the ontogeny of predator-induced vertical migration of copepods. *Nature* **356**, 54-57.

Parejko, K. & Dodson, S. I. (1991). The evolutionary ecology of an antipredator reaction norm: *Daphnia*

pulex and *Chaoborus americanus*. *Evolution* **45**, 1665-1674.

Peckarsky, B. L. (1984). Predator-prey interactions among aquatic insects. In *The ecology of aquatic insects*, 196-254. Resh, V. H. and D. M. Rosenberg (Eds). New York: Praeger.

Pijanowska, J., Weider, L. J. & Lampert, W. (1993). Predator-mediated genotypic shifts in a prey population: experimental evidence. *Oecologia* **96**, 40-42.

Potvin, C. (1993). ANOVA: experiments in controlled environments. In *Design and analysis of ecological experiments*, 46-68. Scheiner, S. M. and Gurevitch, J. (Eds). New York: Chapman & Hall.

Pritchard, G. (1965). Prey capture by dragonfly larvae (Odonata: Anisoptera). *Can. J. Zool.* **43**, 271-289.

Pulliam, H. R. (1988). Sources, sinks, and population regulation. *Amer. Nat.* **132**, 652-661.

Relyea, R. A. & Werner, E. E. (1999). Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* **80**, 2117-2124.

Riessen, H. P. (1992). Cost-benefit model for the induction of an antipredator defense. *Amer. Nat.* **140**, 349-362

Sih, A. (1986). Antipredator responses and the perception of danger by mosquito larvae. *Ecology* **67**, 434-441.

Sih, A. (1987). Predators and prey lifestyles: an evolutionary and ecological overview. In: *Predation: direct and indirect impacts on aquatic communities*, 203-224. Kerfoot, W. C. and Sih, A. (Eds). Hanover: University Press of New England.

Sih, A. (1992). Integrative approaches to the study of predation: general thoughts and a case study on sunfish and salamander larvae. *Ann. Zool. Fennici* **29**, 183-198.

Sih, A. & Kats, L. B. (1994). Age, experience, and the response of streamside salamander hatchlings to chemical cues from predatory sunfish. *Ethology* **96**, 253-259.

Sih, A. & Moore, R. D. (1993). Delayed hatching of salamander eggs in response to enhanced larval predation risk. *Amer. Nat.* **142**, 947-960.

Skelly, D. K. (1992). Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* **73**, 704-708.

Skelly, D. K. (1994). Activity level and the susceptibility of anuran larvae to predation. *Anim. Behav.* **47**, 465-468.

Skelly, D. K. (1995). A behavioral trade-off and its consequences for the distribution of *Pseudacris* treefrog larvae. *Ecology* **76**, 150-164.

Skelly, D. K. & Werner, E. E. (1990). Behavioral and life-historical responses of larval american toads to an odonate predator. *Ecology* **71**, 2313-2322.

Smith, D. C. & Van Buskirk, J. (1995). Phenotypic design, plasticity, and ecological performance in two tadpole species. *Amer. Nat.* **145**, 211-233.

Stauffer, H.-P. & Semlitsch, R. D. (1993). Effects of visual, chemical and tactile cues of fish on the behavioral responses of tadpoles. *Anim. Behav.* **46**, 355-364.

Storfer, A. & Sih, A. (1998). Gene flow and ineffective antipredator behavior in a stream-breeding salamander. *Evolution* **52**, 558-565.

Tollrian, R. & Harvell, C. D. (Eds) (1999). *The ecology and evolution of inducible defenses*. Princeton: Princeton University Press.

Van Buskirk, J. (2000). The cost of an inducible defense in anuran larvae. *Ecology* **81**, 2813-2821.

Van Buskirk, J. & McCollum, S. A. (2000). Functional mechanisms of an inducible defense in tadpoles: morphology and behavior influence mortality risk from predation. *J. Evol. Biol.* **13**, 336-347.

Van Buskirk, J., McCollum, S. A. & Werner, E. E. (1997). Natural selection for environmentally induced phenotypes in tadpoles. *Evolution* **51**, 1983-1992.

Van Buskirk, J. & Schmidt, B. R. (2000). Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. *Ecology* **81**, 3009-3028.

van Tienderen, P. H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**, 1317-1331.

Via, S. & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505-522.

Warkentin, K. M. (1995). Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proc. Nat. Acad. Sci. U. S. A.* **92**, 3507-3510.

Wellborn, G. A., Skelly, D. K. & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst.* **27**, 337-363.

Werner, E. E. (1991). Nonlethal effects of a predator on competitive interactions between two anuran larvae. *Ecology* **72**, 1709-1720.

Werner, E. E. & Anholt, B. R. (1993). Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *Amer. Nat.* **142**, 242-272.

Woodward, B. D. (1983). Predator-prey interactions and breeding-pond use of temporary species in a desert anuran community. *Ecology* **64**, 1549-1555.

Zar, J. H. (1999). *Biostatistical analysis* (Fourth edition). Eaglewood Cliffs: Prentice-Hall International Editions.

Zimmerman, B. L. & Simberloff, D. (1996). An historical interpretation of habitat use by frogs in a central Amazonian forest. *J. Biogeogr.* **23**, 27-46.

Accepted: 6.10.00

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 17-21 (2001)

CANNIBALISM AND KIN DISCRIMINATION IN TADPOLES OF THE AMAZONIAN POISON FROG, *DENDROBATES VENTRIMACULATUS*, IN THE FIELD

KYLE SUMMERS AND REBECCA SYMULA

Department of Biology, East Carolina University, Greenville, NC 27858, USA

Two experiments were conducted to investigate the influence of kinship on aggression and cannibalism in the Amazonian poison frog, *Dendrobates ventrimaculatus*, in eastern Ecuador. Firstly, we placed pairs of kin and pairs of non-kin tadpoles in plastic cups, allowed them to interact over a food item and videotaped their behaviour. The videotapes were analysed for aggressive and associative behaviour. Secondly, we placed pairs of tadpoles in manipulated natural pools in the field, and left them together for one month. The results of the videotaped behavioural experiments did not indicate strongly preferential treatment of kin, although biting was rare in the kin treatments but common in some non-kin treatments. The field experiments indicated that both kin and non-kin tadpoles are likely to be cannibalized if they coexist with larger tadpoles in *Heliconia* pools for a substantial period of time. Ultimately, the study was inconclusive with respect to the occurrence of kin discrimination. However, the study provides important information relevant to the study of kin discrimination by dendrobatid tadpoles in the field.

Key words: cannibalism, kin recognition, *Dendrobates*, behaviour

INTRODUCTION

Kinship is of fundamental importance in understanding the evolution of behaviour (Hamilton, 1964). The study of kin recognition, or how and why animals do or do not recognize and discriminate among kin and non-kin, has received considerable attention (Alexander, 1979; Waldman, 1988; Sherman, Reeve & Pfennig, 1997). Kin recognition among anuran larvae comprises a large part of the literature on kin recognition (Waldman, 1991). Despite frequent demonstrations that anuran larvae can and do recognize kin, the function of such recognition has remained obscure in most cases.

Recent research (Pfennig, Reeve & Sherman, 1993) on tadpoles of the spadefoot toad has demonstrated a clear functional context for kin recognition and discrimination. In this species, there are two tadpole morphs, one of which is highly cannibalistic. Tadpoles were predicted to be more likely to cannibalize non-kin than kin because cannibalizing kin reduces the indirect component of the cannibal's inclusive fitness (Pfennig *et al.*, 1993). This prediction was confirmed: cannibalistic tadpoles prefer to cannibalize non-kin, although cannibals will eat kin and non-kin indiscriminately when the cannibal is hungry, i.e. when its own survival is at risk.

Tadpoles of several species of poison frogs (genus *Dendrobates*) are highly cannibalistic (Wells, 1981; Weygoldt, 1987; Summers, 1990). Hence, this genus is an excellent candidate for investigations of kin recognition in a functional context. In this paper we present the results of laboratory and field experiments on cannibalism and kinship in the Amazonian poison frog, *Dendrobates ventrimaculatus*, from Amazonian Ecuador. The objectives of this study were to: (1) analyse behavioural interactions between related and unrelated pairs of tadpoles to determine if tadpoles discriminate behaviourally on the basis of kinship; (2) investigate the consequences of coexistence in the same pool for related and unrelated pairs of tadpoles.

Dendrobates ventrimaculatus lives in Ecuador, Peru and Brazil. The mating and parental system of *D. ventrimaculatus* from Pompeya in Sucumbios Province in Amazonian Ecuador has been described elsewhere (Summers & Amos, 1997). Briefly, recent field research suggests that this population has male care. Tadpoles are deposited in the pools by males, who carry them on their back from the pool over which they were oviposited (some are simply placed in the pool over which they were oviposited). The tadpoles grow and develop in the pool until metamorphosis, which can require several months in closely related species (Caldwell & Araujo, 1998). Typically, only one tadpole is placed in a pool, but two or more tadpoles are sometimes placed together in the same pool, and a maximum of seven have been found in a single pool (Summers & Amos, 1997; Summers, 1999, unpublished data). Genetic analysis suggests that both related and unrelated tadpoles are placed together (Summers & Amos, 1997). Hence, tadpoles may encounter other tadpoles in the same pool, and these tadpoles may be kin or non-kin.

METHODS

This investigation was carried out in the Quechua village of Limoncocha, and in nearby rainforest near Pompeya, a small Capuchin Mission on the Napo River, in Sucumbios Province, Ecuador, from 23 May to 4 August, 1997. We obtained tadpoles by collecting clutches of eggs in the field and raising them in plastic cups until they hatched. After hatching, the members of the clutch

Correspondence: K. Summers, Department of Biology, East Carolina University, Greenville, NC 27858, USA. *E-mail*: summersk@mail.ecu.edu

were raised in separate plastic cups (one per cup) until placed in an experiment.

We fed at least one member of each clutch *ad libitum* with detritus, mosquito larvae, algae, and (unrelated) *D. ventrimaculatus* eggs. The other members were fed detritus only. After a period of approximately one week to ten days, a substantial difference in mass developed between tadpoles fed *ad libitum* and tadpoles given a restricted diet (mean ± SE: 46±3 mg (large), n=18; 15±1 mg (small), n=18; paired t-test: $t = 9.695$, $P<0.0001$). We also raised unrelated tadpoles in the same way. Tadpoles classified as unrelated were taken from clutches found in plants approximately 10 m or more apart, which is outside of the home range of males and females in this species (Summers & Amos, 1997).

After a substantial difference in mass developed, a large tadpole was placed together with a smaller tadpole in one of two treatments: with kin (presumed full siblings from the same clutch) or with non-kin (unrelated tadpoles from different clutches from different plants). Each related pair of tadpoles was matched with an unrelated pair of tadpoles, and the two treatments (kin and non-kin) were carried out on the same day. Fourteen sets of matched pairs (28 pairs) were used in the experiments. The pairs were matched so that the mass and size differences between the large and small tadpole were as similar as possible between the two treatments (kin and non-kin). There was no significant difference between the two treatments in either the initial mass of the small kin and non-kin tadpoles (paired t-test, n=14, t_{13}=0.306, P=0.7646) or the mass difference between the large and small tadpoles in the kin and non-kin treatments (paired t-test, n=13, t_{12}=1.775, P=0.101).

These matched pairs of tadpoles constituted the matched trials used in the paired tests presented in the results. We placed the two tadpoles of each pair together in a cup with approximately 50 ml of water (equivalent to a small to medium natural pool), and allowed them to acclimate to each other for five to eight hours. A food item (an unrelated egg or embryo) was then placed in the cup and the tadpoles were videotaped for from one half hour to one hour, depending on the availability of electricity. All matched trials were videotaped for the same period of time (so the total amount of observation time was the same for trials with related and unrelated tadpoles), and the data on tadpole interactions were analysed as events per second (e.g. bites per second), to adjust for time length differences between trials.

The behaviour of the tadpoles was scored later from the videotapes by a researcher who did not know the purpose of the experiment, nor which experimental pools contained kin or non-kin. Major categories of behaviour scored were: biting (large tadpole bites the small tadpole), feeding (large or small tadpole feeds on the food item), time spent in contact without aggression (the two tadpoles remain quiescent while in contact or within 1mm of each other), and chasing. Chasing was defined as the large tadpole moving toward the small tadpole, followed by the retreat of the small tadpole. It was not possible to place one large tadpole with one related and one unrelated tadpole simultaneously (e.g. Pfennig *et al.*, 1993), because tadpoles were not sufficiently distinctive in colour or pattern to be individually identifiable, and attempts at marking were not successful.

We carried out further experiments with the kin and non-kin treatments in the field for those experiments that were started more than a month before the end of the study. The day after tadpoles were used in the first experiment (the videotaped behaviour experiment), we placed the tadpoles in *Heliconia* pools in the forest. The pools used form in the leaf and stem axils of *Heliconia* plants, and are the sites most commonly used for breeding by *D. ventrimaculatus* in this area (Summers, 1999).

Placing two tadpoles together replicates the most common type of multiple pool occupancy found in pool surveys: more than two tadpoles in a pool is relatively infrequent (Summers & Amos, 1997). Typically, one tadpole is larger than the other (K. Summers, unpublished observations). We matched the pools for volume for each pair of kin and non-kin. The pools were emptied, any eggs or tadpoles that were found in the pool were removed, and plastic flanges were affixed to the stem of the *Heliconia* plant (with waterproof plastic tape) to prevent adults in the area from using the pools for breeding. The water from each of the matched pools was mixed to equalize the amount of nutrients in the two pools, and the two tadpoles (one large and one small) from each treatment were placed in one of the two pools. We also set up control pools in the same manner, containing only a single small tadpole. These were used as controls for the natural levels of mortality of small tadpoles, without the presence of a large tadpole in the same pool.

The replicate pools were left for one month. The kin and non-kin treatments were fed four eggs over the course of this period, approximating the average availability of eggs in pools that occurs naturally (Summers & Amos, 1997). The control treatments were fed a single egg at the start of the experiment. After one month, the pools were drained and taken apart, and the number of surviving tadpoles was recorded by a researcher who did not know which treatments were which. We are confident that large tadpole remained alive in each pool because the pools were examined frequently (three times a week) and the large tadpole was usually seen in each pool during those inspections. If the large tadpole had died, it would have taken at least a week for the smaller tadpole to reach that size, and the absence of the large tadpole would have been detected during that time period.

This research and associated protocols were approved by INEFAN (the Ministry of Natural Resources of Ecuador): Permit No. 24-IC, and by the Animal Care and Use Committee of East Carolina University: Permit No. D145. Statistical analyses were carried out with

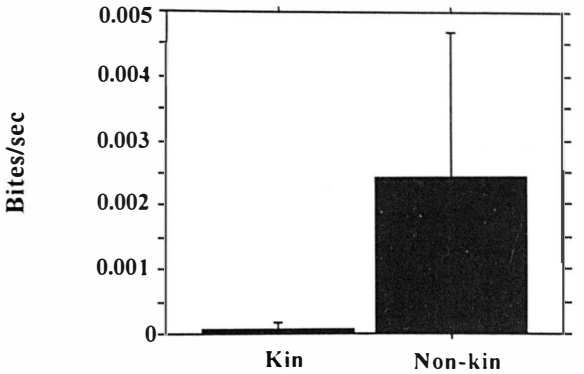


FIG. 1. Number of bites of small tadpole by large tadpole, per second during the experiments. Error bars represent 1 SE.

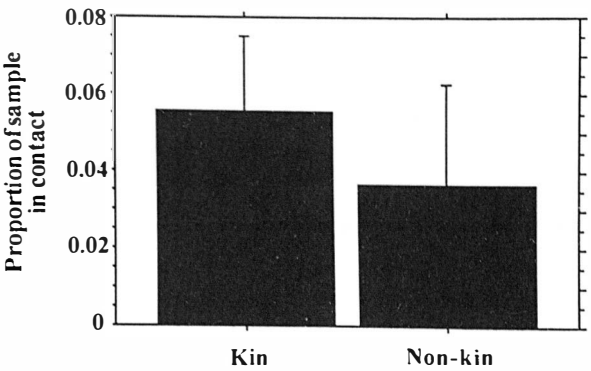


FIG. 3. Proportion of each experiment in which the two tadpoles were in contact or within 1mm of each other.

StatView (Abacus Concepts, 1992). Data were examined for normality and homogeneity of variances, and transformed as appropriate.

RESULTS

Analysis of the videotaped behaviour revealed a tendency for tadpoles to be more aggressive towards non-kin than towards kin. The number of bites per second did not differ between the treatments (Fig. 1, Wilcoxon signed rank test, Z=1.095, n=14, P=0.273), but there was a significant difference between treatments in the variance of this behaviour (F-test, F_{13}=0.001, n=28, P<0.0001). This means that biting between kin was rare, but there was a high variance in the frequency of biting by non-kin. The number of chases per second did not differ between the treatments (Fig. 2, paired t-test, t_{13}= 0.535, n=14, P=0.602). Time spent in contact with each other also did not differ between the treatments (Fig. 3, paired t-test, t_{13}=0.594, n=14, P=0.563). The amount of feeding (number of bites per second) did not differ between the treatments, either for large tadpoles (paired t-test, t_{13}=1.386, n=14, P=0.1890), or small tadpoles (paired t-test, t_{13}=1.515, n=14, P=0.154).

For the field experiments on cannibalism, there were no significant differences among the experimental treatments or the control pools in pool volume (one-way ANOVA, $F_{2,20}$=0.067, n=23, P=0.935). There were no significant differences among the experimental treatments or the control pools in the starting weights of the small tadpoles (one-way ANOVA, $F_{2,22}$=0.056, n=25, P=0.945). Also, there was no significant difference between the kin and non-kin treatments in the mass differential between large and small tadpoles at the start of the field experiments (t-test, t_{16}=0.936, n=18, P=0.363).

There was no difference between the treatments in the mortality of small tadpoles (there was 100% mortality for small tadpoles in both treatments), but there was a significant difference between the mortality of small tadpoles in the two experimental treatments (pooled results) and that of the controls (Fig. 4, Fisher's exact test, P=0.0017). There was no significant difference between the treatments (kin versus non-kin; single tadpole controls were excluded because they started at a different stage) in the growth rates of the surviving (large) tadpoles (t-test, t_{12}=1.341, n=14, P=0.205).

DISCUSSION

The behavioural observations of interactions between kin and non-kin did not demonstrate any dramatic differences between the two treatments. Tadpoles did not chase or bite non-kin significantly more frequently than kin, nor did they spend significantly more time in

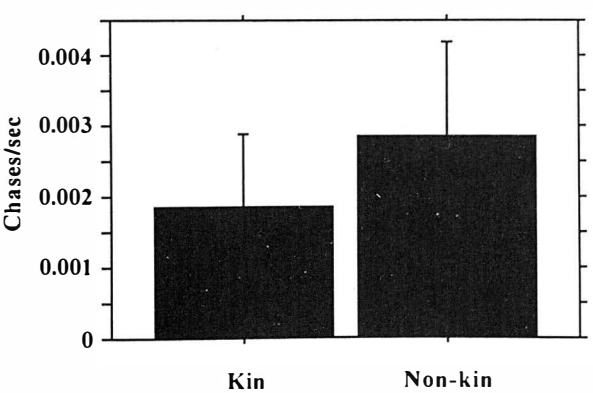


FIG. 2. Number of chases of small tadpole by large tadpole, per second during the experiments. Error bars represent one standard error.

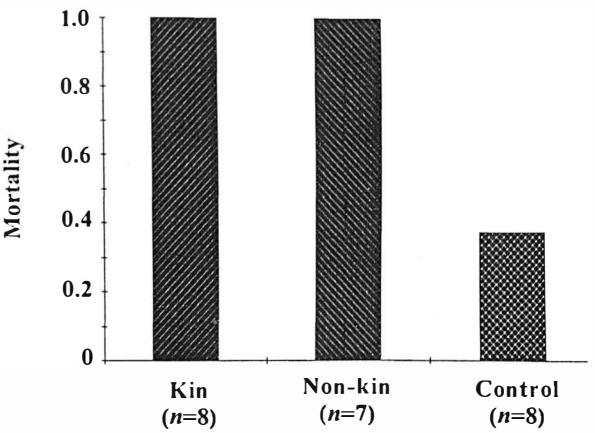


FIG. 4. Proportional mortality in experimental pools in the field, for pools containing pairs of kin, non-kin and controls with a single small tadpole.

passive (non-aggressive) contact with kin than with non-kin. There was no significant difference between kin and non-kin in the amount of feeding done by either large or small tadpoles, so there was no evidence that non-kin tended to monopolize food more, or attempted to prevent the smaller tadpole from eating more vigorously. More complex tests combining different variables (e.g. the number of bites per time spent in contact) also failed to yield significant differences between the kin and non-kin treatments.

However, there was a significant difference between the kin and non-kin treatments in the variance of biting behaviour: biting among kin was consistently rare, whereas the amount of biting between non-kin was variable. This high variance means that the power of the statistical tests to detect preferential treatment was low. Power analysis (Zar, 1996) indicates that to achieve a probability of 75% of detecting a significant difference between the kin and non-kin groups (assuming the mean difference of 0.002 bites per second or 7.2 bites per hour found in this study) would require a sample size of at least 83 trials. Hence, it would be premature to conclude that preferential treatment of kin is absent in this species.

The inconclusive nature of the results of this experiment make it worthwhile to consider potential pitfalls in the methodology that could be corrected by future researchers. We believe that the behaviours we recorded, particularly chasing and biting, are likely to be correlated with tadpole mortality, as has been suggested by other researchers (e.g. Caldwell and Araujo, 1998). However, one possible problem is that the videotaped trials were not long enough. In the field, it may take days or weeks for a small tadpole to succumb to the attacks of a larger tadpole. Hence, the number of interactions observed over the short duration of the videotaped experiments is likely to be relatively small, making detection of significant differences between the kin and non-kin treatments difficult.

The results of the field experiments suggest that, even if there is some tendency to be less aggressive toward kin than toward non-kin, this tendency may usually be insufficient to prevent cannibalism of small tadpoles by larger kin in the field. All of the small tadpoles placed with larger tadpoles were cannibalized, regardless of kinship status. The mortality of small tadpoles in the experimental treatments (kin and non-kin) was significantly higher than that of single small tadpoles in the control treatment, implying that the increased mortality was due to the presence of the larger tadpole. The effect is unlikely to be due simply to starvation as a result of the presence of another tadpole, as the experimental tadpoles were given four times as much food as the single tadpole controls. The effect was also unlikely to be due to water fouling, as single small tadpoles given more than four eggs in feeding experiments did not show high mortality rates (Summers, 1999), and apparently healthy tadpoles were frequently found in pools containing more than four eggs in various stages of decomposition in pool surveys (K. Summers, unpublished observations).

The results presented here suggest that preferential treatment of kin in tadpoles of this species may be less well developed than in the spadefoot toad tadpoles studied by Pfennig *et al.* (1993). Even if this is the case, it does not necessarily mean that these tadpoles do not recognize their kin. Tadpoles may recognize their kin, and yet not discriminate between kin and non-kin behaviourally (Waldman, 1991). This is particularly likely if the costs of altruism (i.e. refraining from cannibalism) are high (Pfennig, 1998).

ACKNOWLEDGEMENTS

We thank Peri Dukes for assistance in the field, Luis Coloma and Alberto Padilla for assistance with research logistics and permits, and Stan Rand, Heather Gray, Susan McRae, David Pfennig, Clive Cummins and two anonymous referees for advice and comments on the manuscript. INEFAN of Ecuador and the Animal Care and Use Committee of East Carolina University provided permits. This research was funded by a grant from East Carolina University.

REFERENCES

Alexander, R. D. (1979). *Darwinism and Human Affairs*. Seattle, Washington: University of Washington Press.

Caldwell, J. P. & Araujo, M. C. (1998). Cannibalistic interactions resulting from indiscriminate predatory behavior in tadpoles of poison frogs (Anura: Dendrobatidae). *Biotropica* **30,** 92-103.

Abacus Concepts (1992). *StatView*. Berkeley, CA: Abacus Concepts.

Hamilton, W. D. (1964). The genetical evolution of social behavior. *Journal of Theoretical Biology* **7,** 1-52.

Pfennig, D. W., Reeve, H. K. & Sherman, P. W. (1993). Kin recognition and cannibalism in spadefoot toad tadpoles. *Animal Behaviour* **46,** 87-94.

Pfennig, D. W. (1998). Kinship and cannibalism. *BioScience* **47,** 667-675.

Sherman, P. W., Reeve, H. K. & Pfennig, D.W. (1997). Recognition systems. In: *Behavioral Ecology: an Evolutionary Approach*, 69-96. Krebs, J. R. & Davies, N.B. (Eds). Oxford: Blackwell.

Summers, K. (1990). Paternal care and the cost of polygyny in the green dart-poison frog, *Dendrobates auratus*. *Behavioral Ecology and Sociobiology* **27,** 307-313.

Summers, K. (1999). The effects of cannibalism on Amazonian poison frog egg and tadpole deposition and survivorship in *Heliconia* axil pools. *Oecologia* **119,** 557-564.

Summers, K. & Amos, W. (1997). Behavioral, ecological and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. *Behavioral Ecology* **8,** 260-267.

Waldman, B. (1988). The ecology of kin recognition. *Annual Review of Ecology and Systematics* **19**, 543-571.

Waldman, B. (1991). Kin recognition in amphibians. In: *Kin Recognition*, 162-219. Hepper, P. (Ed). Cambridge: Cambridge University Press.

Wells, K. (1981). Parental behavior of male and female frogs. In: *Natural Selection and Social Behavior* Alexander, 184-197. Alexander, R. D. & Tinkle, D. W. (Eds). New York: Chiron Press.

Weygoldt, P. (1987). Evolution of parental care in dart poison frogs (Amphibia: Dendrobatidae). *Zeitschrift Zoologische Systematik, Evolution-forsch.* **25**, 51-67.

Zar, J. H. (1996). *Biostatistical Analysis.* Prentice Hall, New Jersey.

Accepted: 18.12.00

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 23-27 (2001)

A GENETIC ASSESSMENT OF BRITISH POPULATIONS OF THE SAND LIZARD (*LACERTA AGILIS*)

TREVOR J. C. BEEBEE AND GRAHAM ROWE

School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG

We investigated sand lizard (*Lacerta agilis*) populations in Britain by genetic analysis across eight polymorphic microsatellite loci. Genetic diversity as determined by mean expected heterozygosity was high in all three distinct regions where the species occurs (Dorset, Surrey and Merseyside), though allelic diversity was lower on Merseyside than in Surrey or Dorset. There was significant genetic differentiation between populations in all three of these widely separated zones, as judged both by Fst and Rst estimators. A genetic test for population bottlenecks confirmed that in at least two of the areas currently inhabited, Surrey and Merseyside, *L. agilis* has undergone substantial recent declines. The significance of these findings for sand lizard conservation is discussed.

Key words: sand lizards, *Lacerta*, conservation, genetics, microsatellites

INTRODUCTION

The sand lizard *Lacerta agilis* is one of only six reptiles native to Britain. It is also one of the two rarest, confined within recent historical times to three small areas of suitable habitat (Smith, 1951): the lowland heaths of Dorset and south-west Hampshire, heathland in the Surrey Weald, and the coastal dunes of Merseyside and North Wales. A major decline in the distribution and abundance of *L. agilis* has followed widespread losses of these critical habitats (e.g. Moore, 1962; Jackson, 1979; Corbett 1988; Webb 1990). Sand lizard populations have also become increasingly fragmented within the three distribution zones. These conditions can lead to genetic depauperization and inbreeding depression, issues that have caused concern among conservation biologists in relation to a wide range of endangered species, including sand lizards (e.g. Frankham, 1996; Olsson, Gullberg & Tegelstrom, 1996; Gullberg, Olsson & Tegelstrom, 1999). Although successful management practices have been developed for *L. agilis* in Britain and the species is given maximum protection under the law (Corbett & Tamarind 1979; Moulton & Corbett, 1999), neither of these important developments will necessarily alleviate any consequences of genetic impoverishment.

Polymorphic microsatellite loci are widely used markers for the study of population genetics in the context of relatively short (ecological) time periods (e.g. Jarne & Lagoda, 1996; Sunnucks, 2000). A suite of *L. agilis* microsatellites was recently developed by Gullberg, Tegelstrom & Olsson (1997) and used to investigate the structure of Swedish sand lizard populations (Gullberg, Olsson & Tegelstrom, 1998). In this paper we describe a study of British sand lizards, using those microsatellite markers to investigate genetic diversity and differentiation among animals living in the three areas where the species currently exists in Britain.

Correspondence: T. J. C. Beebee, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG. E-mail: t.j.c.beebee@sussex.ac.uk

MATERIALS AND METHODS

SAMPLING

Terminal digits from toes were obtained, under licence, from lizards in all three areas of the British distribution (Fig. 1). Toe clipping causes no significant damage to the animal and for many years has been used as a marking procedure for *L. agilis*. Nevertheless, because the species is both rare and endangered the sample sizes were small. Seven Merseyside lizards, eight Dorset lizards and eleven Surrey lizards were toe-clipped during 1999 and the toes were stored in ethanol prior to DNA extraction. The Merseyside lizards and some of the Surrey lizards were maintained in vivaria for captive breeding purposes at the time of sampling, but all were wild-caught animals. None were from sites where there had been previous releases of translocated lizards. The Dorset animals came from two separate heathland sites (four from each). All the lizards were released immediately after sampling, either at the site of capture or back into vivaria.

MICROSATELLITE ANALYSIS

DNA was obtained from each toe by a standard proteinase K digestion, phenol-chloroform extraction and ethanol precipitation procedure. 50-100 ng DNA were used in PCR assays of final volume 20 µl, otherwise as described by Gullberg *et al.* (1997), using α^{33}P- dATP as a radioactive label and with separate primers for each of the microsatellite loci. The PCR products were electrophoresed through 6% polyacrylamide gels, subjected to autoradiography and alleles were scored by reference to M13 sequence markers all as described elsewhere (Rowe, Beebee & Burke, 1997).

DATA ANALYSIS

Conformance to Hardy-Weinberg equilibrium and linkage equilibrium between loci were tested using the computer programs BIOSYS-1 and GENEPOP 3.1

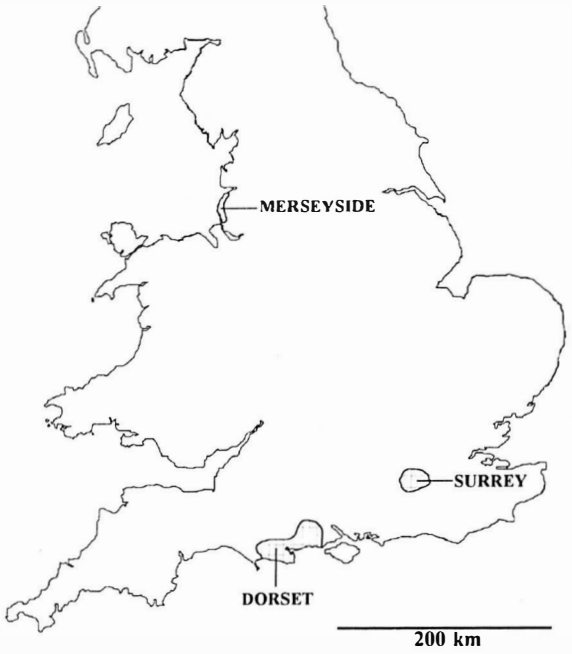


FIG. 1. Distribution of *L. agilis* in Britain showing sampling sites.

(Swofford & Selander, 1981; Raymond & Rousset, 1995). Genetic diversity indices, notably mean number of alleles per locus, percentage of loci polymorphic at the 95% criterion (P^{95}), observed and expected (unbiased) heterozygosities (H_o and H_e respectively) and Cavalli-Sforza chord (D_c) genetic distances (Cavalli-Sforza & Edwards, 1967) were also estimated using BIOSYS-1. Genetic differentiation between populations was measured by pairwise Fst (Weir & Cockerham, 1984) and Rst (Slatkin, 1995) using the programs FSTAT 1.2 and RSTCALC 2.1 respectively (Goudet, 1999; Goodman, 1997). Recent population trends were investigated with the BOTTLENECK program (Piry, Luikart & Cornuet, 1999). Randomization tests were carried out using the program RT version 2.1 (Manly 1997) using 5000 randomizations in two-sample comparisons. Other statistical analyses were performed using the STATISTIX computer package after testing data for normality as appropriate.

RESULTS

Of the 10 microsatellite loci available for study, eight (*La-1, -2, -3, -4, -5, -8, -9* and *-10*) demonstrated consistent polymorphic banding patterns in British *L.*

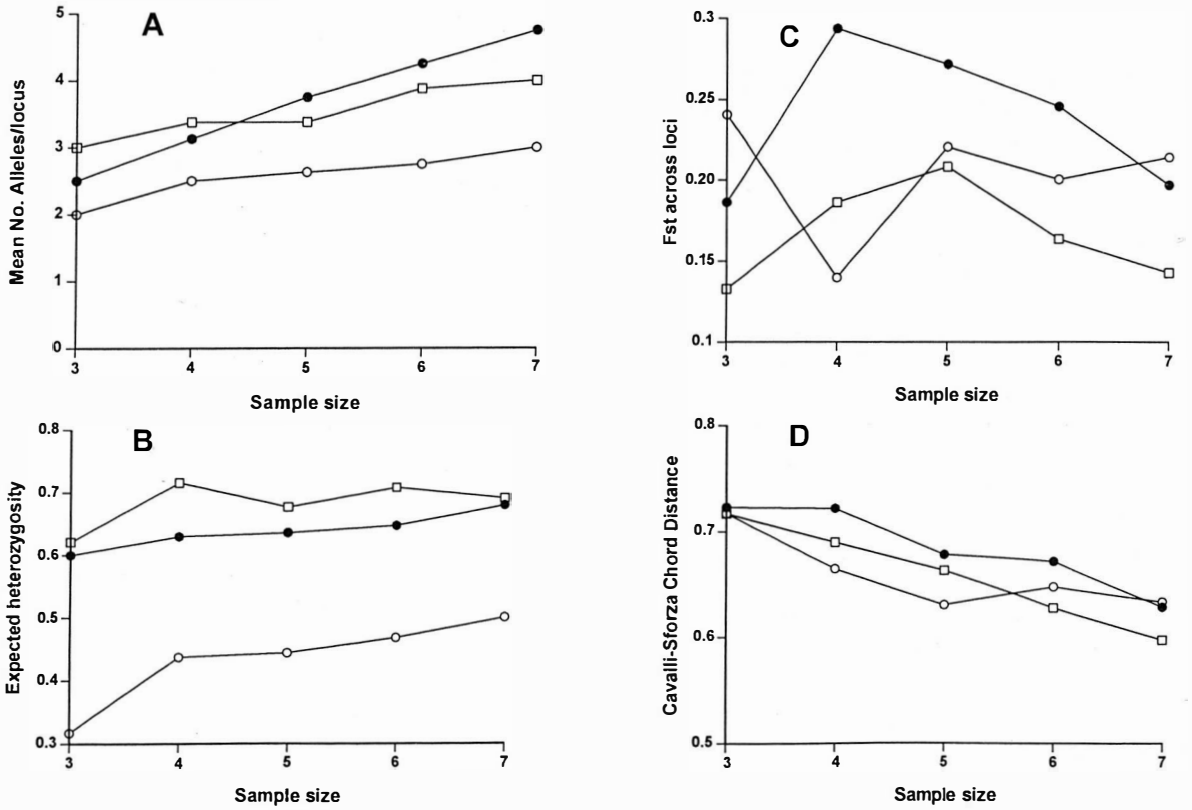


FIG. 2. Sample size dependency of genetic estimators. A: Allele number per locus. Data are averages of five randomly selected groups of each sample size except 7 (for which there was only one possible group in Merseyside). B: Mean expected heterozygosity, H_e. Data are averages of five randomly selected groups of each sample size except 7 (for which there was only one possible group in Merseyside). C: Mean Fst. Data are averages of five random comparisons between some of the randomly chosen groups used in A and B, excepting 7 where there was just one possible comparison. D: Cavalli-Sforza Chord Distance (D_c). Data are averages of five random comparisons between some of the randomly chosen groups used in A and B, excepting 7 where there was just one possible comparison. Solid circles: Dorset population (A & B) or Dorset x Merseyside comparison (C & D); Open circles: Merseyside population (A & B) or Merseyside x Surrey comparison (C & D); Open squares: Surrey population (A & B) or Dorset x Surrey comparison (C & D).

TABLE 1. Genetic variation among *L.agilis* populations. P^{95}, percentage of polymorphic loci at the 95% criterion; H_o, mean observed heterozygosity; H_e, mean expected heterozygosity; SD, standard deviation.

Location	Total alleles	Mean no. alleles/ locus±SD	P^{95}	H_o	H_e
Dorset	39	4.88±0.74	100	0.570	0.678
Surrey	37	4.63±0.68	100	0.549	0.691
Merseyside	24	3.00±0.57	75	0.423	0.500

agilis. *La-6* yielded no PCR products from British sand lizards while *La-7* produced no products from Swedish lizards (Gullberg *et al.* 1997) or from British ones. Dorset and Surrey lizards were polymorphic at all eight loci whereas Merseyside lizards were monomorphic at *La-3* and *La-10*. Only *La-10* in Surrey lizards failed to conform with Hardy-Weinberg expectations and no sets of loci showed significant linkage disequilibrium in any population. The markers were therefore considered appropriate for investigating genetic diversity in the British sand lizard populations.

Because the sample sizes were so small it was important to test the effects of this factor on the various genetic estimators. To do this, random samples of 3-7 individuals were selected from each population and a range of genetic parameters estimated as a function of sample size (Fig. 2). Mean allele numbers per locus and genetic distance (D_c) both showed linear sample-size effects within the range available for analysis. The absolute values of these parameters were therefore meaningless in the present study, but relative comparisons were nevertheless informative. Thus Merseyside allele numbers were consistently lower than those of Dorset and Surrey, whereas genetic distances were consistently similar between all three localities. Randomization tests indicated that Merseyside allele numbers were significantly lower than those in Surrey (P=0.030) or in Dorset (P=0.025) but that there were no differences in this parameter between Surrey and Dorset (P=0.356). By contrast, sample-size dependent trends were weak or non-existent for heterozygosity and differentiation estimators (H_e and Fst respectively), a situation which also held for Rst (data not shown). Estimates of the partitioning of genetic variation (pooling across all loci) indicated that inter regional differentiation (mean Fst=0.191) somewhat exceeded variation within regions (mean Fis=0.116).

TABLE 2. Genetic differentiation of *L. agilis* populations. The probability (P) that Fst or Rst is not significantly different from 0.

Comparison	Fst (P)	Rst (P)
Dorset x Surrey	0.133 (<0.001)	0.165 (0.02)
Dorset x Merseyside	0.186 (<0.001)	0.295 (0.009)
Surrey x Merseyside	0.241 (<0.001)	0.288 (0.001)

Estimates of the sample-size independent parameters for the full data set are shown in Tables 1 and 2. Taken together the data imply that genetic diversity in the Merseyside sand lizards was lower than in Surrey and Dorset lizards, which were broadly similar, though differences in H_e were not statistically significant (Kruskal-Wallis statistic = 2.1359, P=0.3437). Randomization tests of heterozygosity also failed to show any significant differences between regions. Genetic differentiation, however, was substantial between all three regions with both Fst and Rst values significantly different from zero in all pairwise comparisons.

Application of a bottleneck test based on excess heterozygosity relative to allele numbers (Cornuet & Luikart, 1996) supported the inference that there have been substantial recent declines in sand lizard numbers. Despite the fact that sample size was lower than that recommended for adequate statistical power in this test, two of the three areas (Merseyside and Surrey) demonstrated significant heterozygote excess – indicative of bottlenecking – with P=0.023 and P=0.027 respectively.

DISCUSSION

Although sample sizes were small, microsatellite analysis across eight loci has provided useful insights into the British sand lizard populations. Larger samples would have permitted statistically more robust analyses, but in our estimation these would probably not have altered the main conclusions. Genetic diversity was related to population size in the expected way, with the smallest and most isolated population, in Merseyside, demonstrating the lowest diversity of the three British regions. Recent estimates of adult sand lizard population sizes in Merseyside, Surrey and Dorset are in the region of 200-500, <1000 and 6000-8000 adults respectively (Corbett, 1994; Wheeler, Simpson & Houston, 1993). This relationship was unlike the situation in Sweden where, surprisingly, no such correlation between genetic diversity at microsatellite loci and population size was evident (Madsen *et al.*, 2000). Reasons for this difference are unknown, but the Swedish study was at a finer level of scale than ours and there is clearly scope for more detailed analysis of British sand lizard genetics within each region. One consequence of heathland fragmentation, in particular, may be a reduction in the genetic diversity of sand lizards at a local level. However, assuming our data are representative of regional patterns, lizards in all three areas maintained substantial diversity at microsatellite loci. Indeed, British *L. agilis* compare favourably in terms of heterozygosity with Hungarian animals tested across the same loci (mean H_e=0.70) and proved substantially more diverse than Swedish sand lizards with a mean H_e of 0.45 (Gullberg, Olsson & Tegelstrom, 1998).

Sand lizards also make an interesting comparison with natterjack toads (*Bufo calamita*), a species with a

similarly restricted distribution in Britain (Beebee, 1977). Natterjacks on the Merseyside coast were also assayed across eight microsatellite loci and exhibited lower genetic variation than sand lizards in the same area, despite the fact that the current census population size of natterjacks is at least tenfold larger than that of sand lizards (Corbett, 1994; Rowe, Beebee & Burke, 1998; 1999). Thus Merseyside natterjacks (with a sample size of 200) exhibited a mean of only 2.35 alleles per locus, a P^{95} of 62.5% and a mean H_e of 0.295. These differences may stem from the very different population dynamics of lizards and toads, with the latter undergoing larger population fluctuations over short time periods (Beebee, Denton & Buckley, 1996). Unlike lizards, toads have a breeding system in which many individuals in any particular generation probably fail to reproduce successfully (Scribner, Arntzen & Burke, 1997). Effective population sizes (i.e. numbers of animals reproducing successfully averaged over multiple generations) are therefore likely to be much smaller in these amphibians, relative to census sizes, than is the case with lizards. Both of these features are likely to impact on genetic diversity, although other reasons (such as different mutation rates in toads and lizards) could also account for the interspecific differences observed.

The estimators of genetic differentiation in sand lizards revealed significant differences between all three sample areas that are consistent with separation of the three regions at roughly the same time, presumably soon after postglacial colonization when forest development eliminated intervening open habitats (Vincent, 1990). These results also suggest that for conservation purposes populations in the three regions should be considered as distinct clades worthy of protection in their own right.

The genetic bottleneck tests indicated that there have been substantial recent declines of sand lizard effective population sizes in at least two of the three geographical regions. This independent genetic assessment of the fate of British sand lizards accords with conclusions derived from direct field survey (Corbett, 1994; Moulton & Corbett, 1999). There can be little doubt that this species has responded dramatically to the extensive losses of, and damage to, its sensitive heathland and dune habitats, and that conservation measures for it are fully justified.

ACKNOWLEDGEMENTS

We thank the Herpetological Conservation Trust, especially Chris Davies, Nick Moulton and Mike Preston for assistance with sampling, Madryn Lake and Inga Zeisset for assistance with the microsatellite analyses, Bryan Manly for advice about randomization tests, and Tony Gent, Peter Rothery and an anonymous referee for helpful suggestions. The work was carried out under Home Office licence PPL 70/3950.

REFERENCES

Beebee, T. J. C. (1977). Environmental change as a cause of natterjack toad (*Bufo calamita*) declines in Britain. *Biological Conservation* **11**, 87-102.

Beebee, T. J. C., Denton, J. S. & Buckley, J. (1996). Factors affecting population densities of adult natterjack toads *Bufo calamita* in Britain. *Journal of Applied Ecology* **33**, 263-268.

Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis: models and estimation procedures. *Evolution* **21**, 550-570.

Corbett, K. F. (1988). Distribution and status of the sand lizard *Lacerta agilis agilis* in Britain. *Mertensiella* **1**, 92-100.

Corbett, K. F. (1994). *Pilot study for sand lizard UK recovery programme*. English Nature, Peterborough.

Corbett, K. F. & Tamarind, D.L. (1979) Conservation of the sand lizard, *Lacerta agilis*, by habitat management. *British Journal of Herpetology* **5**, 799-823.

Cornuet, J. M. & Luikart, G. (1996). Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**, 2001-2014.

Frankham, R. (1996). Relationship of genetic variation to population size in wildlife. *Conservation Biology* **10**, 1500-1508.

Goodman, S. J. (1997). RSTCALC: A collection of computer programs for calculating unbiased estimates of genetic differentiation and gene flow from microsatellite data and determining their significance. *Molecular Ecology* **6**, 881-885.

Goudet, J. (1999). *FSTAT, a program for IBM PC compatibles to calculate Weir & Cockerham's (1984) estimators of F-statistics (version 1.2)*. Institute of Zoology and Animal Ecology, Lausanne University, Switzerland.

Gullberg, A., Olsson, M. & Tegelstrom, H. (1998). Colonisation, genetic diversity, and evolution in the Swedish sand lizard, *Lacerta agilis* (Reptilia, Squamata). *Biological Journal of the Linnean Society* **65**, 257-277.

Gullberg, A., Olsson, M. & Tegelstrom, H. (1999). Evolution in populations of Swedish sand lizards: genetic differentiation and loss of variability revealed by multilocus DNA fingerprinting. *Journal of Evolutionary Biology* **12**, 17-26.

Gullberg, A., Tegelstrom, H. & Olsson, M. (1997). Microsatellites in the sand lizard (*Lacerta agilis*): description, variation, inheritance, and applicabilty. *Biochemical Genetics* **35**, 281-295.

Jackson, H. (1979). The decline of the sand lizard *Lacerta agilis* L. population on the sand dunes of the Merseyside coast, England. *Biological Conservation* **16**, 177-193.

Jarne, P. & Lagoda, P. J. L. (1996). Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution* **11**, 424-429.

Madsen, T., Olsson, M., Wittzell, H., Stille, B., Gullberg, A., Shine, R., Andersson, S. & Tegelstrom, H. (2000). Population size and genetic diversity in sand lizards (*Lacerta agilis*) and adders (*Vipera berus*). *Biological Conservation* **94**, 257-262.

Manly, B. F. J. (1997). RT: A program for randomisation testing. Version 2.1. Western Ecosystem Technology Inc., Wyoming, USA.

Moore, N. W. (1962) The heaths of Dorset and their conservation. *Journal of Ecology* **60**, 369-391.

Moulton, N. & Corbett, K. F. (1999) *The Sand Lizard Conservation Handbook*. English Nature, Peterborough.

Olsson, M., Gullberg, A. & Tegelstrom, H. (1996). Malformed offspring, sibling matings, and selection against inbreeding in the sand lizard (*Lacerta agilis*). *Journal of Evolutionary Biology* **9**, 229-242.

Piry, S., Luikart, G. & Cornuet, J. M. (1999). BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**, 502-503.

Raymond, M. & Rousset, F. (1995). GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Rowe, G., Beebee, T. J. C. & Burke, T. (1997) PCR primers for polymorphic microsatellite loci in the anuran amphibian *Bufo calamita*. *Molecular Ecology* **6**, 401-402

Rowe, G., Beebee, T. J. C. & Burke, T. (1998). Phylogeography of the natterjack toad *Bufo calamita* in Britain: genetic differentiation of native and translocated populations. *Molecular Ecology* **7**, 751-760.

Rowe, G., Beebee, T. J. C. & Burke, T. (1999). Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. *Animal Conservation* **2**, 85-92.

Scribner, K. T., Arntzen, J. W. & Burke, T. (1997). Effective number of breeding adults in *Bufo bufo* estimated from age-specific variation at minisatellite loci. *Molecular Ecology* **6**, 701-712.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457-462.

Smith, M. A. (1951). *The British Amphibians and Reptiles*. Collins, London.

Sunnucks, P. (2000). Efficient genetic markers for population biology. *Trends in Ecology & Evolution* **15**, 199-203.

Swofford, D. L. & Selander, R. B. (1981). BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 281-283.

Vincent, P. (1990). *The Biogeography of the British Isles*. Routledge, London.

Webb, N. R. (1990) Changes on the heathlands of Dorset, England, between 1978 and 1987. *Biological Conservation* **51**, 273-286.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Wheeler, D. J., Simpson, D. E. & Houston, J. A. (1993). In: *The Sand Dunes of the Sefton Coast*. Ed. D. Atkinson & J. Houston. National Museums and Galleries, Merseyside.

Accepted: 12.1.01

SHORT NOTES

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 29-32 (2001)

PARENTAL CARE BEHAVIOUR IN *LEPTODACTYLUS PODICIPINUS* (COPE, 1862) (ANURA, LEPTODACTYLIDAE)

ITAMAR A. MARTINS

Departamento de Biologia – Laboratório de Zoologia, Universidade de Taubaté, São Paulo, Brazil

Key words: parental care, anurans, *Leptodactylus*

In anurans, parental care is known in about 10% of the species and occurs in one form or another in 14 families (Salthe & Mecham, 1974; McDiarmid, 1978; Wells, 1977, 1981). Parental care, which requires some investment, may be present in either males or females and may be considered an adaptation to specific ecological conditions (Wells, 1977, 1981). McDiarmid (1978) recognized 12 categories of parental behaviour in anurans, based on oviposition habitat, the site of parental care, the nature of larval stage and the sex of the caring parent. Wells (1981) proposed four broader categories: (1) caring for eggs, (2) caring for tadpoles, (3) transporting eggs, and (4) transporting tadpoles, excluding cases in which ovoviviparity or viviparity occurs.

Aquatic care of eggs and tadpoles is rare among anurans, probably because the habitats of adults and tadpoles are different (McDiarmid, 1978; Wells, 1981). Only a few cases of parental care in species with aquatic reproduction have been reported, mainly in species with foam mass nests and free-living tadpoles. This phenomenon is more often present in tropical species with terrestrial reproduction and may represent an evolutionary response to predation in aquatic environments (Salthe & Mecham, 1974; Crump, 1974; Wells, 1981).

One common characteristic of the subfamily Leptodactylinae is egg-laying in a foam mass either floating at the water's surface or in burrows (Downie, 1996). Few reports of egg-care exist for the family Leptodactylidae. However, care of aquatic eggs and tadpoles has been observed in adults of two species of the "*ocellatus*" species group (Heyer, 1969), *Leptodactylus ocellatus* (Vaz-Ferreira & Gehrau, 1975) and *Leptodactylus bolivianus* (Wells & Bard, 1988), and in two species of the "*melanonotus*" group (Heyer, 1969), *Leptodactylus validus* and *L. leptodactyloides* (Downie, 1996). The present paper reports parental caring behaviour in *Leptodactylus podicipinus*.

Correspondence: I. A. Martins, Departamento de Biologia – Laboratório de Zoologia, Universidade de Taubaté – UNITAU, CEP: 12020-270, Taubaté, São Paulo, Brazil. *E-mail:* istama@uol.com.br

Field observations were made between August 1994 and December 1995 in Fazenda Santa Maria, 2 km from Nova Itapirema district (21° 1' S, 49° 4' W), in north-western São Paulo State, Brazil. The observations were made in a permanent pond (40 m length, 6 m width and 0.8 m depth), in an open area with marginal and emergent, grassy vegetation (Poaecea and Cyperacea). The "focal animal" method (Martin & Bateson, 1986) was employed, with weekly observations made from 1600 hrs to 0200 hrs, using flashlights and, when necessary to avoid disturbing the animals, red filters. Additional daily visits (n=32) were made to observe the daytime behaviour of females in relation to eggs and tadpoles.

Each adult specimen found (60 male and 25 female) was captured and marked according to Martof's (1953) technique of toe amputation. Small egg samples were collected and fixed in 5% formalin. The spawning places were marked with stakes which were numbered with reference to the numbers of the corresponding females. The stakes served as reference points to follow the female displacements (i.e. movements from one place to another).

Each female with eggs and/or tadpoles was observed during three daily periods of 30 mins each. During each period the following data were collected: number of pumping motions by the female, time elapsed from one pumping motion to the next, time from one displacement to another and total displacement distance. At the end of each period a stake was used to mark the last resting place of each female and her tadpoles. At each visit, the distance from the last resting place to that observed was measured. Small tadpole samples from each female were collected, killed and preserved in 10% formalin for later identification of larval stages.

The following description is based on observations of 11 females. The female laid her eggs in a foam nest within the male's territory. After egg-laying, the male abandoned the spawning site and moved to another. The female caring behaviour began at spawning. She remained beside or partially under the foam nest during the whole embryonic development period, with the head directed towards the mass, but never on it. Occasionally, females were observed capturing small spiders and ants on the egg mass; this behaviour was easily observed as the female did not quit her eggs even in the presence of an observer.

When the hatchlings began to leave the foam nest and became free-living, the female remained most of the time with her body partially submerged, under or beside the foam nest. As the larvae abandoned the foam mass and fell into the water they formed a dense shoal near the female's body. The tadpole shoals were found at depths between 3 cm and 19 cm, near the margins, among emergent water plants. The female remained close to the tadpoles and the tadpoles scraped the female's postero-dorsal region and hind legs.

The tadpoles always followed the female, who made bouts of pumping motions (*sensu* Wells & Bard, 1988)

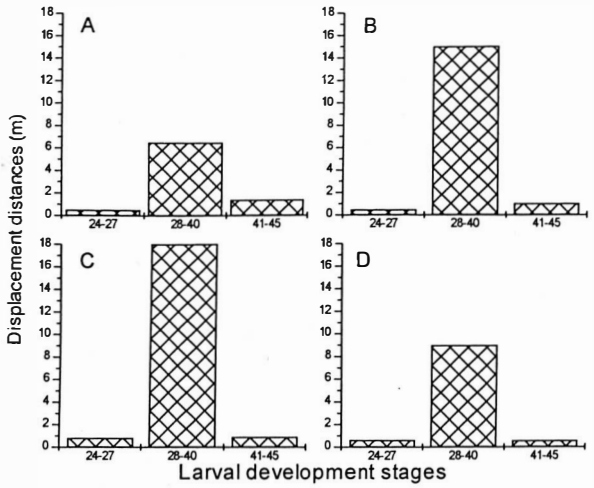


FIG. 1. Displacement distances of four female *Leptodactylus podicipinus* and their tadpoles in relation to larval development stages. Females: A (n=25), B (n=26), C (n=32) and D (n=28); n= number of the observations.

of the posterior region of the body at the water surface before moving from one place to another. In the pumping motion the female arched her back and alternately lowered and raised the hind legs and the vent above the water's surface, producing waves which reached the tadpoles. The pumping movements were rhythmic and usually, when a series of more than six was produced, the last movements were faster and had a larger amplitude than those previous. Soon after the female moved, the tadpoles moved towards her, taking the same route. When most of the tadpoles reached the female, she moved again and made a new series of pumping motions. Sometimes the female paused between displacements until all the tadpoles reached her and made contact with her body. The number of pumping movements made by a female during one displacement ranged from 1 to 39 and showed no correlation with either the distance travelled (r=0.117, P>0.10, n=63) or the time elapsed between one series of pumping movements and the next (r=0.081, P>0.10, n=63). The distances travelled in each displacement ranged from 10 cm to 80 cm (mean±SD = 29.8±15.8 cm, n=63). The total displacement of females and their tadpoles ranged from 6.5 m to 18 m.

The distance travelled by the females with their tadpoles showed a positive correlation with the stage of larval development (r_s=0.846, P<0.01, n=11). In the first few days after the larvae left the foam mass, the female displacements were short, about 10-45 cm away from the spawning site (Fig. 1). One female remained stationary with the tadpoles at the spawning site for six days. The longest displacements were observed in females whose tadpoles were in stages 28 through 40 (Gosner, 1960). The displacement distances were short, – 2 metres at most – at later stages (42 through 44), the female then remaining in shallow water (3-8 cm) at the pool margins. At the final stages (Gosner stages 44 and 45) the distance between the female and the tadpoles became greater again and the female was often observed out of the water, on the vegetation. There was no contact between female and tadpoles in the latter period, suggesting that the parental influence on the offspring had ended. The average duration of larval development, and consequently of parental care by the female, was 28 days (SD = ±3.7 days; range = 25 to 32 days, n=11).

Pumping followed by displacement was observed in all females with tadpoles (n=11), and usually began about 30 mins after sunset. In broad daylight, the females remained at more protected sites under the vegetation and the tadpoles were similarly less active, although they kept removing sediments and feeding. The displacements were mostly unidirectional, but on a number of occasions (n=28) the female moved forward, made the pumping movements, returned to the tadpole shoal, made new pumping movements, and proceeded to the site where she had first made the movements. Most often, this occurred when the female had travelled a long distance or was in water more than 20 cm deep. At other times the female seemed to return to the shoal to reunite the dispersed tadpoles, which had formed two or three separate shoals.

Trivers (1972) considered parental care to be "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring".

Parental care is present in some form in the majority of anuran families and this taxonomic distribution suggests several independent evolutionary origins within the order (Salthe & Mecham, 1974). In many anurans parental care is restricted to the females (Wells, 1977). Work on the genus *Leptodactylus* demonstrates an adaptive tendency towards female parental care (Wells & Bard, 1988; Downie, 1996).

Many leptodactylid species have developed evolutionary mechanisms that protect the offspring from harmful agents (Vaz-Ferreira & Gehrau, 1974). In the genus *Leptodactylus*, the more common mechanisms in this connection are: (1) a floating foam nest; (2) a burrow with the eggs and embryos in foam; (3) a floating foam nest with the female caring for the eggs and tadpoles until metamorphosis (Heyer, 1969; Vaz-Ferreira & Gehrau, 1975; Duellman & Trueb, 1986; Wells & Bard, 1988; Downie, 1996).

Spawning in foam protects the eggs and embryos from desiccation and from aquatic and terrestrial predators (Heyer, 1969; Villa, McDiarmid & Gallardo, 1982). In *L. podicipinus*, the presence of foam in addition to parental care constitutes a specialized reproductive pattern, as found in other species of *Leptodactylus* (Vaz-Ferreira & Gehrau, 1975; Wells & Bard, 1988; Downie, 1996). The presence of the female near the foam nest in *L. podicipinus* may be interpreted as a defence mechanism against small predators, such

as arthropods, but may also be a necessary prelude to caring for the tadpoles.

Comparing the types and modes of egg-laying in foam masses, Heyer (1969) concluded that the groups "*melanonotus*" and "*ocellatus*" share the primitive spawning pattern for *Leptodactylus*. This reproductive pattern is probably present in other species of both groups, but awaits confirmation. Egg-care is a part of the complex parental care of the leptodactylids (Weygoldt, 1987). The presence of the female with the egg mass, as observed in both groups, may be an adaptation to the circumstance in which spawning occurs within the male territory. The presence of the female may hinder the males from occupying the egg-laying site as a calling site.

Among the types of parental care present in Leptodactylidae, the groups "*ocellatus*" and "*melanonotus*" show one more line of specialization in caring for the offspring, involving the protection of eggs, embryos and tadpoles as well as communication through stereotyped signals, revealing an adaptive novelty in relation to the rest of the family.

Vaz-Ferreira & Gehrau (1975) suggest that the function of the type of parental behaviour described here is protection against predators, such as birds and fish. Downie (1996) tested this hypothesis in *L. validus* and demonstrated that this function may be viable for larger species such as *L. ocellatus* and *L. bolivianus*, but not for the smaller and less aggressive species such as those of the "*melanonotus*" group. The fact that during the day female *L. podicipinus* and their tadpoles shelter under the vegetation suggests some form of protection against diurnal predators. The presence of females near the eggs and tadpoles may not assure protection against large predators, but it may well confer protection against small predators such as some invertebrates. In the present study, some females were observed preying upon small insects and spiders that were near or on the eggs and, a few times, attacking spiders that attempted to prey upon tadpoles. In addition, it is possible that the displacements by females and tadpoles to more varied and sheltered sites function as a strategy for avoiding attack by large predators.

The parental care behaviour observed in *L. podicipinus* is similar to those described by Wells & Bard (1988) and Downie (1996), in which the females similarly remain with the eggs, attend the tadpole shoals up to metamorphosis and make pumping movements. The pumping of the posterior region of the body at the water surface is a complex form of physical and/or chemical communication between females and aquatic larvae that allows the females to lead their tadpoles through the pool (Wells & Bard, 1988). Vaz-Ferreira & Gehrau (1975) did not report pumping motions by female *L. ocellatus*, but indicated that the waves used for communication in that species were produced by low-frequency vocalizations. Thus, there appear to be two very different mechanisms involved in producing waves that seem to play a part in communication in the "*ocellatus*" and "*melanonotus*" groups.

The similarity of this kind of parental care behaviour in two species groups ("*ocellatus*" and "*melanonotus*") reinforces Heyer's (1969) hypothesis that the two groups are the most closely related of the five groups of *Leptodactylus* species (Downie, 1996). It is possible that this form of parental care occurs also in other species of both groups, especially in "*melanonotus*".

Acknowledgements. I thank Dr Jorge Jim and Dr Celio F. B. Haddad for their suggestions and opinions. I am grateful to Dra. Elieth F. Spirandeli Cruz for providing valuable comments. Dr. Arif Cais and Dra. Denise de C. Rossa- Feres provided suggestions and help in field work. Dr. Nelson Bernardi helped with the English version. CAPES provided financial support.

REFERENCES

Crump, M. L. (1974). Reproductive strategies in a tropical anuran community. *Univ. Kans. Mus. Nat. Hist. Misc. Publ.* **61**, 1-68.

Downie, J. R. (1996). A new example of female parental behaviour in *Leptodactylus validus*, a frog of the leptodactylid "*melanonotus*" species group. *Herpetological Journal* **6**, 32-36.

Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians*. New York: McGraw-Hill, 670p.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetológica* **16**, 183-190.

Heyer, W. R., (1969). The adaptative ecology of the species group of the genus *Leptodactylus* (Amphibia, Leptodactylidae). *Evolution* **23**, 421-428.

Martin, P. & Bateson, P. (1986). *Measuring behaviour - an introductory guide*. Cambridge: Univ. Press.

Martof, B. S. (1953).Territoriality in the Green frog, *Rana clamitans*. *Ecology* **34**, 165-174.

McDiarmid, R. W. (1978). Evolution of parental care in frogs. In: *The Development of Behavior. Comparative and Evolutionary aspects*, 127-147. Burghardt, G. M. & Beckoff, M. (Eds). Garland Publishing, Inc., New York.

Salthe, S. N. & Mecham, J. S. (1974). Reproductive and courtship patterns. In: *Physiology of the Amphibia*, II. Academic Press, 309-400. Lofts, B. (ed.). Academic Press, New York.

Trivers, R. L. (1972). Parental investment and sexual selection. In: *Sexual selection and the descent of man 1871-1971*, 136-179. Cambell, B. C. (ed). Aldine Publishing Company, Chicago.

Vaz-Ferreira, R. & Gehrau, A. (1974). Proteccionde la prole en Leptodactylidos. *Revista de Biologia del Uruguay* **v. II**, 59-62.

Vaz-Ferreira, R. & Gehrau, A. (1975). Comportamiento epimeletico de la rana comun, *Leptodactylus ocellatus* (Amphibia, Leptodactylidae). I. Attencion de la cria y actividades alimentarias y agresivas relacionades. *Physis* **34**, 1-14.

Villa, J., McDiarmid, R. W & Gallardo, J. M. (1982). Arthropod predators of Leptodactylid frog foam nests. *Brenesia* **19/20**, 577-589.

Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* **25**, 666-693.

Wells, K. D. (1981). Parental behavior of male and female frogs. In: *Natural Selection and Social Behavior: Recent Research and New Theory,* 184-197. Alexander, R. D. & Tinkle, D. W. (eds). Univ. Michigan, Michigan Press.

Wells, K. D. & Bard, K. M. (1988). Parental behavior of an aquatic-breeding tropical frog. *Leptodactylus bolivianus. Journal of Herpetology* **22**, 361-364.

Weygoldt, P. (1987). Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). *Z. Zool. Syst. Volut.-forsh* **25**, 51-67.

Accepted: 3.11.00

HERPETOLOGICAL JOURNAL, Vol. 11, pp. 33-35 (2001)

SEASONAL VARIATIONS OF THE DIET OF *LAUDAKIA STELLIO* (AGAMIDAE) FROM NISYROS ISLAND, DODECANESE (GREECE)

PIETRO LO CASCIO[1], CLAUDIA CORTI[2] AND LUCA LUISELLI[3]

[1]*Museo di Storia Naturale dell'Università di Firenze, Sezione di Zoologia "La Specola", via Romana 17, I-50125 Florence, Italy*

[2]*Dipartimento di Biologia Animale e Genetica, Università degli Studi di Firenze, via Romana 17, I-50125 Florence, Italy*

[3]*Centro di Studi Ambientali "Demetra", Rome, Italy and Museo Civico di Storia Naturale, piazza Aristide Frezza 6, Capranica Prenestina, Rome, Italy*

Key words: lizard diet, faecal analysis, agamid, Greece

Laudakia stellio is a large-sized agamid lizard with a scattered distribution in mainland and island Greece, north-western Africa, and south-western Asia (Arnold, Burton & Ovenden, 1978). In the Dodecanese archipelago the species is widespread, but it is not found on the smaller islands (Chondropoulos, 1986; Foufopoulos, 1997). The first record for Nisyros island, our study site, dates back to Zavattari (1929).

The data available on the ecology and natural history of *L. stellio* are very few, possibly because of its extremely elusive habits. Apparently, *L. stellio* shares some important natural history features with some of its African relatives of the genus *Agama* (e.g. *A. agama*, cf. Harris, 1964; Yeboah, 1982; Anibaldi, Luiselli & Angelici, 1998), including bright male dorsal coloration which can be changed rapidly, brightest colours in dominant specimens, and peculiar displays (exaggerated posturing, head bobs, etc) (Arnold *et al.*, 1978; Xyda, 1986). Almost nothing is documented on the diet of *L. stellio*, whereas detailed data are available for the common rainbow lizard *A. agama* of Africa (Chapman & Chapman, 1964; Harris, 1964; Cloudsley-Thompson, 1981; Anibaldi *et al.*, 1998). Some information is available from Cyprus (Cecconi, 1908) and Antiparos (Cyclades; Cattaneo, 1984), and a general review is found in Beutler (1981).

In this note we present detailed data on the composition of the diet of a population of *L. stellio* from the Dodecanese, based on food remains in faecal pellets. We focus our attention on seasonal variations in diet composition.

Correspondence: Luca Luiselli, Institute Demetra, via dei Cochi 48/B, I-00133 Rome, Italy. *E-mail*: lucamlu@tin.it

The fieldwork was conducted by one of us (PLC), during spring (April and May) and summer (August) 1999 on Nisyros island, Dodecanese, Greece (36° 35' N, 27° 10' E). Nisyros is a medium-sized island (41.2 km^2, maximum elevation 698 m a.s.l.) belonging to the active volcanic arc of the southern Aegean. The origin of the island can be dated back to 150 000 years ago, when there was very strong eruptive activity in the region (Vougioukalakis, 1998). The soil consists mainly of tephritic-pumiceous material coming from the last eruptions. The vegetation of Nisyros consists of high Mediterranean evergreen maquis comprising the following species: *Quercus macrolepsis, Quercus coccifera, Pistacia terebinthus* and *Olea europaea sylvestris*. The total human population of the island is approximately 1000 and is mainly concentrated on Mandraki village, where *L. stellio* is known as "Kurkutavlos". In the study area, *L. stellio* is sympatric with the lacertid *Ophisops elegans*, and possibly with the snake *Coluber gemonensis* (Boettger, 1888; Ghigi, 1929). Potential predators of *L. stellio* could be raptors, feral cats and rats. *Ophisops elegans* is undoubtedly the only other lizard of Nisyros island (Boettger, 1888; Ghigi, 1929; Lo Cascio, unpublished data).

Sampling was carried out on four occasions during the spring and two occasions during the summer. Faeces were collected on the surface of dry, stony walls that represent the typical habitat of *L. stellio* in the study area. The collected faeces could undoubtedly be attributed to the study species on account of their typical size and shape. Indeed, the size of the collected faeces was too large for the smaller species *Ophisops elegans*. Moreover, *Laudakia stellio* and *Ophisops elegans* exhibited a clear habitat separation, with the former species inhabiting only stony walls, whereas the latter is an exclusively ground-dwelling species (P. Lo Cascio, unpubl. data). The transects of walls where the faeces were collected were 100 m to 200 m long, and the local density of *L. stellio* specimens averaged one specimen every 5-10 m of linear transect (P. Lo Cascio, unpubl. data). The lizard population size within the walked transects was estimated to consist of 100 to 400 individuals (P. Lo Cascio, unpubl. data). Along these transects, however, sightings of faecal pellets were scarce, mainly because in parts of the transect it was impossible to search for them due to the thick spiny bush coverage. Faeces were examined in the laboratory under a dissecting microscope. Faecal analysis has proved to be a reliable technique for evaluating diet composition of large lizards (Angelici, Luiselli & Rugiero, 1997; Anibaldi *et al.*, 1998). Remains were identified to the lowest taxon possible. Size of prey items (precision ± 1 mm) was evaluated by comparisons with reference collections of Nisyros arthropods and seeds stored in the Zoological Museum "La Specola" (Florence, Italy) and in the private collection of one author (P. Lo Cascio). For practical reasons, every food remnant was assigned to one of the following seven size categories:

(1) 0-3 mm, (2) 3.1-6 mm, (3) 6.1-9 mm, (4) 9.1-12 mm, (5) 12.1-15 mm, (6) 15.1-18 mm, (7) >18.1 mm in length. Statistical analyses were computed using SPSS for Windows PC package, with alpha set at 5% and all tests being two-tailed.

In total 81 faecal pellets were obtained from the soil, 54 during spring and 27 during summer. Given that we did not capture *L. stellio* specimens, and given the highly sedentary habits of agamid lizards inhabiting walls (e.g. see Anibaldi *et al.*, 1998), it remains possible that several pellets were produced by single specimens. These faeces consisted of 857 identifiable food remains during spring (mean = 15.9 identifiable food items per pellet), and 127 identifiable food remains during summer (mean = 4.7 identifiable food items per pellet). The mean number of prey per pellet was significantly different between spring and summer (one-way ANOVA: F=43.132, df=1,78, P<0.00001). However, the spring faeces were not larger than the summer ones, which were often drier and more fragmented due to faster desiccation. In addition, small stones and remains of leaves (presumed to be secondarily ingested by lizards) were found in a few faeces, but are not included in this analysis. The dietary data are summarized in Table 1. The diet composition shifted considerably from spring to summer (χ^2 test, 2 x 19 contingency table, P<0.000001). During spring, it consisted mainly of arthropods, though gastropods, seeds and fruits were occasionally consumed. If we consider only arthropod remains, there was a considerable excess of Coleoptera (particularly Rutelidae) and Hymenoptera (mainly Formicidae) over all other taxonomic groups (χ^2 test, df=6, P<0.000001). During summer, arthropods (mainly insects) were also frequently eaten, but plants and seeds assumed a much higher significance. In particular, the fruits of *Pistacia terebinthus* were frequently consumed (Table 1). In this regard, it is noteworthy that *L. stellio* specimens were observed while searching actively for these fruits on the ground, whereas they were never observed climbing on *Pistacia* trees. This is consistent with Beutler's (1981) report that *L. stellio* is not arboreal in the Aegean islands. With regard to *Pistacia* fruits, it should be mentioned that they are mainly constituted by the seed, while the edible part is limited to a thin external layer where the available energy content is concentrated.

TABLE 1. Dietary composition by numbers of items (N), and by numbers of pellets containing that prey type (n), in samples of *Laudakia stellio* faecal pellets from Nisyros island, Dodecanese (Greece).

	Spring		Summer	
Prey Type	*N*	*n*	*N*	*n*
(1) PLANTS AND SEEDS				
Plant remains	1	1	-	-
Compositae seeds	37	16	-	-
Pistacia terebinthus fruits	-	-	40	21
Undetermined seeds	12	2	-	-
(2) ANIMALS				
Gastropoda (Pulmonata)	4	4	1	1
Acarina	1	1	-	-
Araneae	7	7	4	4
Heteroptera undet.	20	17	7	5
Odontoscelis sp.	1	1	-	-
Dermaptera	-	-	7	7
Orthoptera (Acridoidea)	2	2	2	2
Coleoptera undet.	10	10	22	5
Tenebrionidae	12	11	4	4
Chrysomelidae	2	2	-	-
Nitidulidae	4	4	-	-
Curculionidae	22	17	7	5
Lixus sp.	2	1	-	-
Carabidae	6	5	-	-
Scarabaeidae	1	1	-	-
Cetoniidae	2	2	-	-
Oxythyrea cinctella	3	3	-	-
Rutelidae	31	10	-	-
Anisoplia sp.	7	4	-	-
Blitopertha lineolata	465	44	1	1
Buprestidae	-	-	1	1
Lepidoptera	1	1	-	-
Hymenoptera (Chrysididae)	2	2	2	2
Apoidea	39	18	3	1
Formicidae	163	29	26	11

Size was determined in nearly 95% of the total sample of consumed items during spring, and 62.2% of consumed items during summer. During spring, there was a unimodal size distribution peaking at 9-12 mm (Fig. 1). This modal size corresponded with the size of Rutelidae beetles, which accounted for over 80% of consumed items in this size category. During summer, the modal size of the arthropods eaten was slightly smaller (Fig. 1), but the seed component was much higher than in spring.

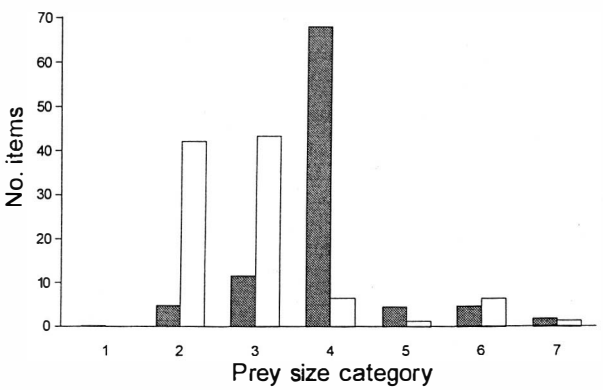


FIG. 1. Prey size distributions inferred from faecal pellets of *Laudakia stellio* from Nisyros island, Dodecanese (Greece). Total N is 813 during spring and 79 during summer. Shaded columns indicate spring, and unshaded columns indicate summer. Symbols for prey size categories: 1, 0-3 mm; 2, 3.1-6 mm; 3, 6.1-9 mm; 4, 9.1-12 mm; 5, 12.1-15 mm; 6, 15.1-18 mm; 7, >18 mm

In general, our data show that there is a very pronounced seasonal dietary shift in the studied population of *L. stellio.* During the spring, these lizards are typical arthropod-eating agamids. In this period, they exhibited a foraging tactic that may be described as "slow searching scan behaviour", which is quite similar to a typical sit-and-wait strategy. It is noteworthy that Rutelidae, as well as other flower-visiting Scarabeoidea beetles that are the commonest prey items in spring, are readily available in the environment in that season. In fact, they exhibit a peculiar phenology that is limited exclusively to the early spring months (April to May). During summer, *L. stellio* forages upon arthropods of similar size, but actively searches for larger fruits and seeds. In this regard, it should be noted that the plant *Pistacia terebinthus* has the fruit phase in summer, and is therefore not available to lizards during spring. Thus, it is concluded that the lizards exhibited a mixed foraging strategy in summer, with an active searching component. The modal size of animal prey was slightly greater in *L. stellio* during spring than during summer, but this may reflect differences in prey size availability in the various study sites and during the survey periods (cf. Vicente, Araujo & Barbault, 1995). The fact that Agamidae species may feed upon both animal and plant material is not, in itself, a new finding, as it has already been mentioned for African species (Harris, 1964).

Cecconi (1908) reported a diet based on arthropods (mainly insects) for a few dissected specimens of this species, and Beutler (1981) also reported generically the presence of beetles and orthopterans in the diet. These studies provide data quite similar to those recorded by us in spring. Conversely, Cattaneo (1984) found that invertebrates (mainly small beetles) are not the only dietary components of *L. stellio,* as flowers of Compositae plants (mainly *Chrysanthemum coronarium*) are also consumed. It is noteworthy that the evolution of herbivory is positively correlated with insularity in the primarily insectivorous lizards of the family Lacertidae (Pérez-Mellado & Corti, 1993; Van Damme, 1999), and our data on *L. stellio* suggests that the same may be true for Old World Agamidae as well.

Acknowledgements. We are grateful to Ornella Mammoliti and Thomas Watson for their help during the field trips, and to Francesco M. Angelici (FIZV, Rome), Massimo Capula (Zoological Museum, Rome), and an anonymous reviewer for helpful comments on the manuscript.

REFERENCES

Angelici, F. M., Luiselli, L. & Rugiero, L. (1997). Food habits of the green lizard, *Lacerta bilineata*, in central Italy and a reliability test of faecal pellet analysis. *Ital. J. Zool.* **64**, 267-272.

Anibaldi, C., Luiselli, L. & Angelici, F. M. (1998). Notes on the ecology of a suburban population of rainbow lizards in coastal Kenya. *Afr. J. Ecol.* **36**, 199-206.

Arnold, E. N., Burton, J. A. & Ovenden, D. W. (1978). *Field guide to the Reptiles and Amphibians of Britain and Europe*. Glasgow, Collins.

Beutler, A. (1981). *Agama stellio* (Linnaeus, 1758) - Hardun, pp. 161-177. In: Boehme, W. (ed), *Handbuch der Reptilien und Amphibien Europas*. Wiesbaden, Akademische Verlagsgesellshaft.

Boettger, O. (1888). Verzeichnis der von Herrn E. von Oertzen aus Griechenland und aus Kleinasien mitgebrachten Batrachier und Reptilien. *Sitz. Akad. Wiss. Berlin* **5**, 139-186.

Cattaneo, A. (1984). *Podarcis erhardii naxensis* ad Antiparos (Cicladi centrali) e note di campagna sull'erpetocenosi dell'isola (Reptilia). *Atti Soc. ital. Sci. Nat. Mus. Civ. St. nat. Milano* **125**, 245-254.

Cecconi, G. (1908). Intorno al nutrimento dell' *Agama stellio* L. *Boll. Mus. Zool. Anat. Comp. R. Univ. Torino* **23**, 1-2.

Chapman, B. M. & Chapman, R .F. (1964). Observations on the biology of the lizard *Agama agama* in Ghana. *Proc. Zool. Soc. Lond.* **143**, 121-132.

Chondropoulos, B. P. (1986). A checklist of the Greek reptiles. 1. The lizards. *Amphibia-Reptilia* **7**, 217-235.

Cloudsley-Thompson, J. L. (1981). Bionomics of the rainbow lizard *Agama agama* (L.) in eastern Nigeria during the dry season. *J. Arid Envir.* **4**, 235-245.

Foufopoulos, J. (1997). The reptile fauna of the Northern Dodecanese (Aegean Islands, Greece). *Herpetozoa* **10**, 3-12.

Ghigi, A. (1929). Ricerche faunistiche nelle isole italiane dell'Egeo. Risultati generali e conclusioni. *Arch. Zool. Ital.* **13**, 293-354.

Harris, V. A. (1964). *The life of the rainbow lizard.* London, Hutchison.

Pérez-Mellado, V. & Corti C. (1993). Dietary adaptation and herbivory in lacertid lizards of the genus *Podarcis* from western Mediterranean islands (Reptilia: Sauria). *Bonn. Zool. Beitr.* **44,** 193-220.

Van Damme, R. (1999). Evolution of herbivory in lacertid lizards: Effects of insularity and body size. *J. Herpetol.* **33**, 663-674.

Vicente, L. A., Araujo, P. R. & Barbault, R. (1995). Ecologie trophique de *Podarcis bocagei berlengensis* et de *Lacerta lepida* (Sauria, Lacertidae) sur l'Ile de Berlenga (Portugal). *Rev. Ecol. (Terre et Vie)* **50**, 317-351.

Vougioukalakis, G. E. (1998). *Blue volcanoes: Nisyros.* Nisyros Regional Council, 78 pp.

Yeboah, S. (1982). Observations on territory of the rainbow lizard, *Agama agama. Afr. J. Ecol.* **20**, 187-192.

Zavattari, E. (1929). Ricerche faunistiche nelle isole italiane dell'Egeo. Anfibi e Rettili. *Arch. Zool. Ital.* **13**, 31-36.

Xyda, A. (1986). Supplementary evidence on the biometry and ecology of the lizard *Stellio stellio* of Greece and Cyprus. *Biologia Gallo-hellenica* **12**, 451-458.

Accepted: 1.12.00

BOOK REVIEW

Pythons of Australia. A Natural History. Geordie Torr (2000). 112 pp. University of New South Wales Press/ Krieger Publishing Co., Florida. US$ 24.50 (paper).

The python fauna of Australia is one of the earth's most spectacular snake faunas, consisting of 13 species ranging in length from about 0.5 m (*Antharesia perthensis*) to around 5 m (*Morelia amethystina*). These fascinating snakes have been subjected to intense scientific research in the last two decades, mainly by Rick Shine and his associates at the University of Sydney, and have become popular subjects of research. In addition, python natural history has been covered in some excellent regional books on Australian reptiles, including for example, Cogger (1982) and Shine (1991). Nevertheless, Torr's *Pythons of Australia* is the first attempt to gather together much of the diverse qualitative and quantitative information on the natural history of these splendid snakes.

The book is organized into seven chapters, covering practically every aspect of Australian python natural history, from fossil history to anatomy and physiology; from behavioural ecology to reproduction and life-history traits; and from conservation to captive breeding. Moreover, the book presents comprehensive species accounts with descriptions and distribution maps for every species of Australian python, and a short bibliography.

The book has many strong points. It is beautifully illustrated by 34 excellent colour photos showing pythons in a number of different postures, including – for example – feeding, ovipositing, hatching, etc. Moreover, the book has plenty of diagrams, generally redrawn from primary research papers, that are used to illustrate the main arguments examined. The most important aspect of this book is that it examines virtually every aspect of the natural history of Australian pythons, with a style and language that can be easily understood by non-professional herpetologists. However, the text is clearly biased towards ecological topics, with taxonomic and systematic aspects only briefly reported upon.

The geographic limitation of the subject matter does, at times, lead to a sense of frustration. Although the focus is on Australian pythons, comparisons with pythons from elsewhere in the world would have enlightened the text. Such comparisons would have been very interesting and useful, because some important aspects of python natural history are repeated in different species from different continents. For instance, large pythons from Asia, Australia, and Africa show remarkable adaptability to habitats disturbed by humans (e.g. compare data in Shine & Fitzgerald, 1996, and in Luiselli, Angelici & Akani, 2000), and this aspect is important for any conservation considerations on a global perspective. Equally, the fact that males are more arboreal than females is a trait that Australian *Morelia spilota* share with Nigerian *Python regius* (Luiselli & Angelici, 1998), and perhaps with other species as well.

The list of references is very short, and almost exclusively comprises work by Shine and associates (19 out of 25 entries!). I feel that a more exhaustive bibliographic coverage would have better served professional herpetologists.

In conclusion, apart for some minor shortcomings, this book is very good, and is a must for all those that are interested in snake natural history or the biology of pythons. Considering the quality of both printing and colour plates, the price of the book represents very good value for money.

REFERENCES

Cogger, H. G. (1981). *Reptiles and Amphibians of Australia.* Sydney: Reed Books.

Luiselli, L. & Angelici, F. M. (1998). Sexual size dimorphism and natural history traits are correlated with intersexual dietary divergence in royal pythons (*Python regius*) from the rainforests of southeastern Nigeria. *Italian Journal of Zoology* **65**, 183-185.

Luiselli, L., Angelici, F. M. & Akani, G. C . (2000). Food habits of *Python sebae* in suburban and natural habitats. *African Journal of Ecology* **39**, in press.

Shine, R. (1991). *Australian Snakes: A Natural History.* Sydney: Reed Books.

Shine, R. & Fitzgerald, M. (1996). Large snakes in a mosaic rural landscape: the ecology of carpet pythons *Morelia spilota* (Serpentes: Pythonidae) in coastal eastern Australia. *Biological Conservation* **76**, 113-122.

Luca Luiselli
Institute Demetra, Rome

Sea Snakes (2nd Edition). Harold Heatwole. (1999). 166 pp. Krieger Publishing Co., Malabar, Florida. US$ 29.50 (paper)

Although I have been studying snakes for more than thirty years, I have focused my attention mainly on temperate-zone species. As a result, there are a number of groups of snakes with which I am relatively unacquainted. Save for a few *Pelamis* I saw floating on the Sea of Cortez in early 1981, sea snakes are one of those groups. Somehow, to my disadvantage, I also never got around to reading very much about them. I therefore looked forward with enthusiasm to reading this new edition of Harold Heatwole's book.

This is a slim volume, providing an overview and general introduction to the sea snakes. Ten chapters cover such topics as distribution and diversity, various aspects of sea-snake ecology, physiological adaptations to the marine environment, and venoms and snakebite. An appendix details the distribution of all the world's sea snakes and references for further reading are given

for each chapter at the end of the book; this ample list of references, ranging from books to journal articles, includes items from the 1920's to the late 1990's. References are not generally provided directly in the text, but sources are given for figures. The book is illustrated with black-and-white photographs, graphs, and line drawings in each chapter, with 29 good-quality colour plates in the middle. The figures are generally clear, although the complete range of natricines is difficult to see in Fig. 2.1, as are the white arrows in Fig. 8.6; there is no scale given in Fig. 3.12, Fig. 6.2 has an unexplained dashed line, and Fig. 2.5 is simply hard to read.

Heatwole uses a liberal definition of sea snakes. Rather than restricting himself simply to the hydrophiids (which make up the bulk of species anyway) and the laticaudids, as many authors might, he essentially includes all species of snakes known to live in brackish or salt water. This definition incorporates several species of colubrids, mainly homalopsines, but also three species of North American natricines, and one species of acrochordid. Conversely, he excludes one hydrophiid and one laticaudid that occur exclusively in freshwater; another mainly marine laticaudid apparently also occurs in one freshwater lake (p. 51). I might add that at least two species of garter snakes (*Thamnophis*) on the west coast of North America are known to forage in salt water, but they are not mentioned in this book; like the other natricines noted above, they barely qualify as "sea snakes" in any case. Natricines aside, all the other species of sea snakes are tropical in distribution and the highest diversity of them is in Australia. This book evidently was (originally, at least) part of a series on the natural history of Australia, where the author worked while studying sea snakes. As a result, the book has a strong focus on Australia, but adequate attention is paid to the rest of the sea-snake world.

This book is technically quite well written and produced; in fact, I had to read almost to the very end to find a minor typographical error on p. 128 ("fisherman" for "fishermen"). However, I must admit that I found the writing uninspiring in places, especially the early part of the book. Chapter 2, on distributions, is a good example, although that particular subject matter perhaps does not lend itself well to much excitement. The definition of "natural history" on p. 30 is also very cumbersome. Heatwole does bring in his personal experiences, but without much emotion or liveliness; the chapters on ecology simply did not grip me as I had hoped they would. However, Heatwole picks up steam in the latter part of the book. The last four chapters are not only informative, but an engaging read. Chapter 8 on diving adaptations really brings the reader into the scientific process of eliminating hypotheses one by one. Chapter 9 on venoms is about as good a popular treatment of this subject, in general, as I have read, including a nice discussion of predator-prey evolutionary "arms races". My only complaint about these last few chapters is that Chapter 7, on physiological adaptation to marine life, is too short.

Although I may have quibbles about the writing of the first part of the book, I learned plenty. I was unaware, for example, of freshwater hydrophiids and laticaudids. I also did not know about light-sensitive tails (p. 45) or specialized feeding on fish eggs by *Emydocephalus annulatus* (p. 48). The discussion of the apparent immunity of *Pelamis* against predation (p. 54) was mainly new to me and I obviously had not paid sufficient attention to the interesting lung structure of acrochordids (p. 80). In this way, the book is very rewarding. However, Heatwole never makes clear to whom he is aiming this book. It reads like a popular book, but its general readability might be enhanced by using less unexplained jargon. Obviously, respect for the reader involves the notion that he/she can use a dictionary, but Heatwole is not very consistent; he defines "eurytopic" (albeit 8 pages after its first appearance in Table 2.1), "nocturnal", "diurnal", and "crepuscular", but not "subtended", "sympatric", "chromatogram", or "standard deviation".

Heatwole obviously "knows his stuff" when it comes to sea snakes, but I must take issue with at least a couple of his more general statements about snakes. For example, the notion of a "continuum" of reproductive modes between oviparity and viviparity (p. 35) is a bit misleading in that it glosses over the fact that in squamates in general – and certainly in snakes – there is effectively a bimodal distribution of periods of egg retention, with a real dearth of intermediate forms. Similarly, as far as I know, there is no evidence that snakes "dislocate" their jaws while swallowing large prey (p. 103), despite popular wisdom on this subject. A reference as old as Wright and Wright (1957) for nomenclature of natricines (p. 8) is not really up-to-date in 1999 (especially given that *Nerodia* in that work is treated as *Natrix*). In any case, it is inevitable that some of the information in this book will be overtaken by subsequent research. For example, a paper published last year suggests that the fossil genus *Pachyrhachis* may not be closely related to mosasaurs after all, contary to what is indicated on p. 8. Such is scientific life: everything we write is yesterday's news.

All said, I am glad I read this book. It may not have been completely satisfying, but it certainly whetted my appetite.

Patrick T. Gregory
University of Victoria

THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS

(revised July 2000)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: *Full Papers* (no length limit); *Reviews* and *Mini-reviews* (generally solicited by a member of the editorial board); *Short Notes*; controversies, under *Forum* (details available from the Editor); and *Book Reviews*. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2. *Three* copies of all submissions, and illustrations, should be sent to the Scientific Editor. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigour, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer, and double-spaced with wide margins all round. The journal is typeset direct from the author's computer diskette, so all manuscripts should be prepared using a wordprocessor (preferably on a PC-compatible microcomputer). If figures are prepared using computer graphics, they should be supplied separately and NOT embedded in the text of the wordprocessor file. Preferred formats are MS Word for Windows (text) and MS Excel, Bitmap, TIFF, or JPEG files (graphics). It is not necessary to submit a computer diskette with the initial manuscript, but this will be required in the event of the manuscript being accepted for publication.

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. A *sans serif* font (e.g. Universe or Helvetica) is preferred.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal.

   Bellairs, A. d'A. (1957). *Reptiles*. London: Hutchinson.

   Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

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

   Dunson, W. A. (1969*b*). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American J. Physiol.* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer diskette ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

10. Proofs should be returned to the Managing Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

THE HERPETOLOGICAL JOURNAL

Volume 11, Number 1 2001

CONTENTS

Herpetological Journal vol. 10, no. 4 was published on 23 February 2001