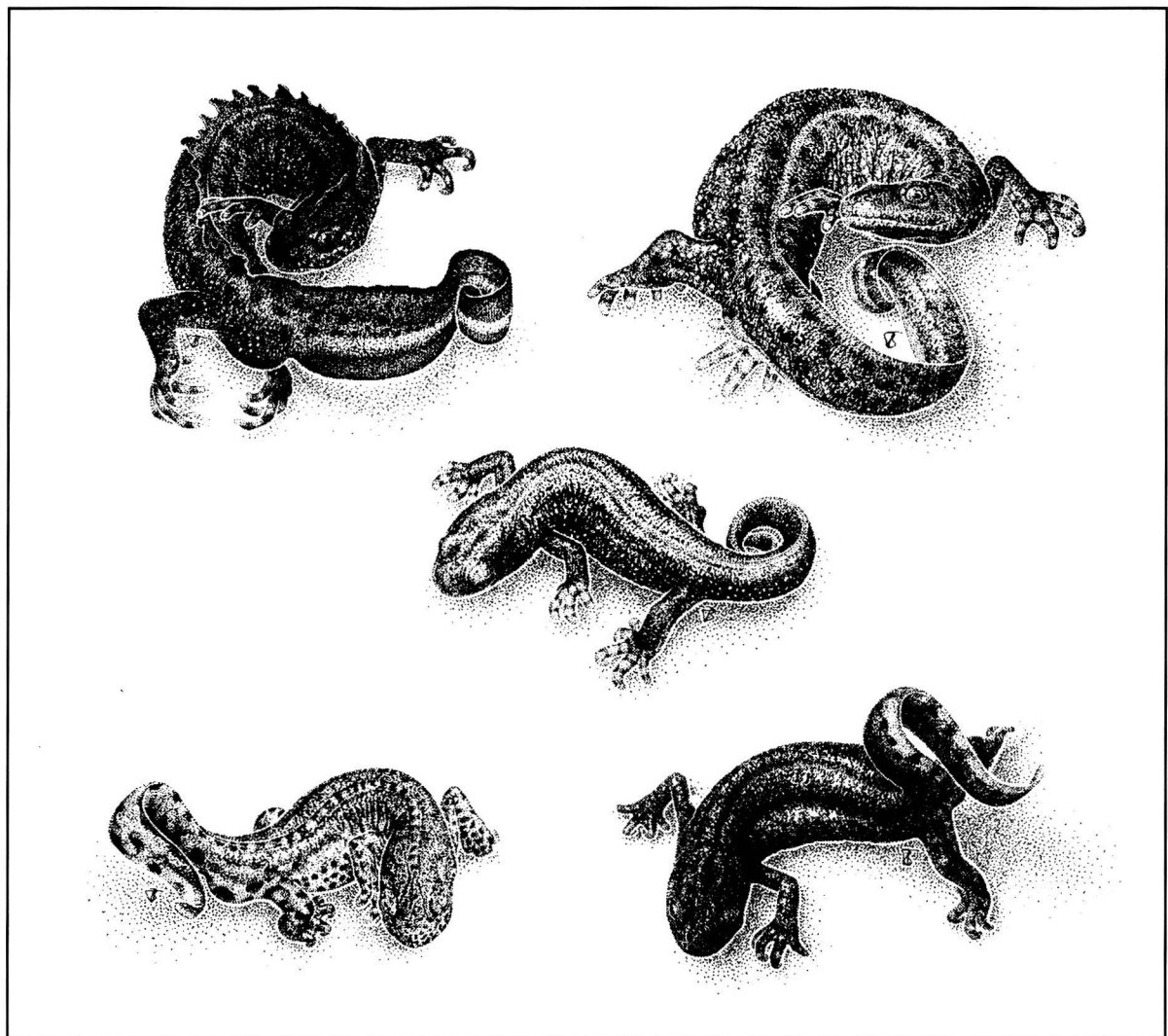


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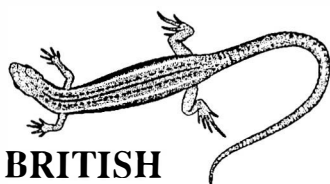
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HABITAT-STRUCTURAL AND METEOROLOGICAL PARAMETERS INFLUENCING THE ACTIVITY AND LOCAL DISTRIBUTION OF THE GOLDEN-STRIPED SALAMANDER, *CHIOGLOSSA LUSITANICA*

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We studied the activity pattern and small-scale spatial distribution of the golden-striped salamander, *Chioglossa lusitanica*, in a forest plot in northwestern Portugal. A large proportion of the local population inhabited an underground watercourse and foraged in the woodland, leaving and entering the underground shelter through three holes. Surface activity – measured as the number of salamanders out in the open and their distance from the inferred shelter – was positively associated with relative humidity and precipitation. A negative relationship between dispersion distance and temperature was found in females. Distances moved were higher for adult salamanders than for juveniles. Spatial models indicated that males, females and juveniles differed in habitat utilization. The frequency of occurrence of salamanders at the surface was highest in the area of the stream (adults and juveniles), near dry-stone walls (males and juveniles), in areas with a higher than average density of trees (adults) and in areas of dense undergrowth (females). The results suggested that (1) the underground watercourse served as a retreat from which the salamanders would visit the surface, (2) dry stone walls functioned as retreats rather than as foraging grounds, and (3) trees and dense vegetation served as shelter for foraging salamanders. The results are compared with those for phylogenetically related and morphologically similar species.

Key words: *Chioglossa lusitanica*, distribution, habitat, spatial model, Portugal

INTRODUCTION

The distribution of the golden-striped salamander, *Chioglossa lusitanica*, is restricted to the northwestern part of the Iberian Peninsula. Its range corresponds to areas of low and medium altitude (< 1200 m) with high annual precipitation (> 1000 mm; Arntzen, 1981; Vences, 1997). The species lives alongside mountain brooks with well-vegetated margins and has nocturnal habits. Secluded places such as mine galleries are used for reproduction. In habitats like these the natural history of the species is well documented (Goux, 1957; Gonçalves, 1962; Arntzen, 1981, 1994a, 1995; Faria *et al.*, 1996; Vences, 1990, 1993; Teixeira *et al.*, 1999). A picture has emerged in which the ecological requirements and habits of *C. lusitanica* are similar not only to those of its sister-species *Mertensiella caucasica* from the Caucasus, but also to some Nearctic streamside salamanders to which it is phylogenetically unrelated (Hairston, 1987; Tarkhishvili & Serbinova, 1993; Tarkhishvili, 1994; Titus & Larson, 1995; Veith *et al.*, 1998). The understanding of this apparent ecological (and etho-morphological) evolutionary convergence would be advanced by the description for all of these species of ecological profiles under a wide range of environmental conditions. Here we contribute to this growing data

set with observations on *C. lusitanica* in an atypical habitat – a woodland plot with an underground watercourse – by analysing the effects of structural habitat characteristics and weather conditions on the activity and micro-distribution of the species.

MATERIALS AND METHODS

Observations were made in a 0.5 ha area near Alfena (12 km NE of Porto, Portugal) covered with *Pinus pinaster*, *Quercus robur* and *Eucalyptus globulus*, some *Q. suber*, *Salix* sp. and *Alnus glutinosa*, and with an undergrowth dominated by *Osmunda regalis*, *Ulex europaeus*, *Hedera helix*, *Lonicera periclymenum*, *Rubus* sp. and various graminæ. The site is crossed in a north-south direction by two low, dry-stone walls about 50 cm high, and diagonally by an underground stream that discharges into a man-made reservoir (Fig. 1). The watercourse is open to the outside world at the pond edge (Fig. 1: point W) and at three further sites (Fig. 1: X, Y and Z). The holes X and Y are narrow (ca. 30 cm), washed-out crevices along tree roots. Holes W and Z are wide, derelict, man-made entrances. The study area was divided into 72 quadrangular areas (8 m x 8 m), for each of which we counted the number of trees (N_{tree}) and measured the shortest distance from the centre of the grid cell to the dry stone wall (D_{wall}), to the water (pond or position of the watercourse in a straight line, D_{water}), and to the holes (D_{holes}). The undergrowth was classified as vegetation type A - low, grassy vegetation providing

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poor cover, type B - medium-height *Osmunda regalis* and grassy vegetation providing medium cover, or type C - high and dense grassy and herbaceous vegetation with tree shoots, providing good cover. The places where salamanders were found were classified as (1) at an exit hole, (2) at the base of – or within – a dry-stone wall, (3) at the base of a tree, or (4) out in the open.

Twenty-eight rounds of observations at regular intervals of about two weeks were made from 16 March 1995 to 28 March 1996. Temperature and relative humidity were measured locally at the start of each visit, three hours after sunset. Precipitation data (rain fall in mm over 48 hours, measured at 0900 hrs prior to the study night) were obtained from the meteorological station at Matosinhos, 15 km west of Alfena. One search took from two to four hours to complete, depending on time of year, weather conditions, and catch. Orientation was done by visual triangulation with 220 mapped trees as reference points. Accessible parts of the underground watercourse were inspected five times in autumn. Salamanders with snout-vent length of 38 mm or more were considered to be adults and their sex was determined from the expression of secondary sexual characteristics (cf. Arntzen, 1981).

The distribution of the salamanders was analyzed by stepwise linear regression, with relative density (number per square averaged over visits, N_v) as the dependent variable and D_{hole} , D_{wall} , D_{water} , N_{tree} , and vegetation type (VT) as independent variables. The resulting models were visualized with the help of a Geographic Information System (IL WIS 2.0). The activity of the salamanders was estimated through the parameters 'catch' and 'dispersion'. Catch was the total number of salamanders caught during each visit (N_v). Dispersion (m) was the mean shortest distance between each salamander and the structural feature(s) (i.e. exit-hole, dry-stone wall, and watercourse) selected in the distribution models.

The normality of data distribution was tested by Shapiro-Wilk's W -test. Parametric and non-parametric statistical tests followed Sokal & Rohlf (1981) and Siegel & Castellan (1988).

RESULTS

Out of 1550 observations of salamanders above ground, 217 (14%) were juveniles and 1333 (86%) were adults. Excluding salamanders for which gender could not be determined unequivocally, 674 were males (60%) and 454 were females (40%). The observed sex-ratio was male-biased during summer, autumn and winter (G -test of goodness of fit, $15.6 < G < 28.1$, $df=1$, $P < 0.001$), but not significantly different from unity during spring ($G=0.5$, $df=1$, $P > 0.05$). From 130 to 180 salamanders were observed in the underground watercourse in autumn. We observed six pairs in amplexus, and 11 eggs that were deposited just above the water surface.

Fig. 2 shows how N_v (i.e. the no. individuals observed) varied seasonally and in relation to meteorological conditions. A significant relationship

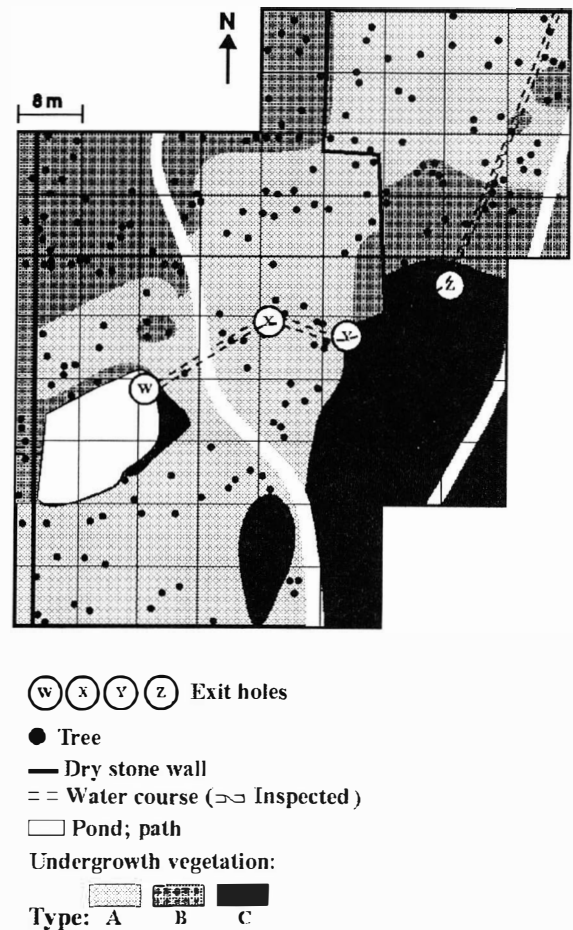


FIG. 1. Map of the area in which *Chioglossa lusitanica* was studied. For a description of the vegetation types see text.

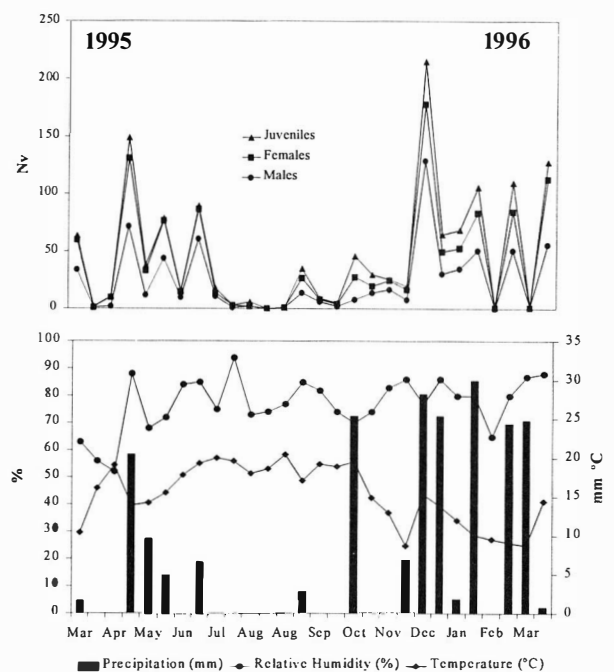


FIG. 2. Numbers of adult and juvenile *Chioglossa lusitanica* observed. Temperature and relative humidity were recorded locally; precipitation was recorded at the 'Estação de Avisos Agrícolas de Entre Douro e Minho' at Matosinhos, 15 km west of the study area. Three sampling periods were conducted in August.

TABLE 1. Average number and dispersion of *Chioglossa lusitanica* observed under dry and moist weather conditions.

	Precipitation in preceding 2 days		Relative humidity	
	No (n=13)	Yes (n=15)	< 95% (n=13)	> 95% (n=15)
<i>Number</i>				
Juveniles	1.9	12.8	2.6	13.7
Males	5.2	40.5	8.0	42.6
Females	2.8	27.8	6.4	27.5
<i>Dispersion (m)</i>				
Juveniles	0.4	1.9	0.3	2.3
Males	1.2	5.2	1.4	5.5
Females	2.4	6.7	2.3	7.5

was observed between N_v for males, females and juveniles with relative humidity (Spearman rank-correlation test, $0.72 < r_s < 0.77$, $P < 0.001$) and with precipitation ($0.60 < r_s < 0.72$, $P < 0.001$), but not with temperature ($-0.35 < r_s < 0.29$, $P > 0.05$). The average catch was higher during visits preceded by precipitation than during dry periods and higher when relative humidity was high ($> 95\%$) than when it was not high (Table 1). It should be noted that rainfall and humidity data were correlated ($r_s = 0.71$, $P < 0.001$). Dispersion did not differ significantly between males and females (Mann-Whitney U -test, $Z = 0.81$; $P > 0.05$) but was higher for adults than for juveniles ($Z = 2.44$, $P < 0.05$). A significant association was observed between dispersion of both adults and juveniles with precipitation ($0.52 < r_s < 0.64$, $P < 0.01$) and relative humidity ($0.65 < r_s < 0.76$, $P < 0.001$). Dispersion was higher during visits preceded by precipitation than during dry periods and higher when relative humidity was high than when it was not high (Table 1). A significant negative relationship of dispersion with temperature was observed for females ($r_s = -0.51$, $P < 0.05$), but not for males or juveniles ($-0.21 < r_s < -0.25$, $P > 0.05$). The two sexes were not found in equal proportions in different microhabitats: males were found more frequently at the bases of trees than females (G -test for independence,

$G = 8.4$, $df = 3$, $P < 0.05$); similarly, adults were found at the bases of trees more frequently than juveniles ($18.1 < G < 36.6$, $df = 3$, $P < 0.001$; Table 2). During wet conditions, adults and juveniles were found relatively often at the bases of trees, while during dry conditions they were found more frequently at the exit holes (G -test for independence, $25.8 < G < 123.0$, $df = 3$, $P < 0.001$; Table 2).

A preliminary analysis showed a poor fit of spatial models based on linear regression ($0.15 < r^2 < 0.30$). However, our observations indicated that hole W was rarely used, and excluding that hole from the analyses markedly improved the fit of the models – for juveniles: $\log(N_s + 1) = 0.781 - 0.010 \times D_{\text{wall}} - 0.022 \times D_{\text{water}}$ ($P < 0.01$, $r^2 = 0.35$); for males: $\log(N_s + 1) = 1.190 - 0.010 \times D_{\text{wall}} - 0.032 \times D_{\text{hole}} + 0.091 \times N_{\text{tree}}$ ($P < 0.01$, $r^2 = 0.64$); for females: $\log(N_s + 1) = 0.443 - 0.022 \times D_{\text{water}} + 0.079 \times N_{\text{tree}} + 0.123 \times TV$ ($P < 0.01$, $r^2 = 0.28$).

The spatial distribution of salamanders as described by these models is presented in Fig. 3.

DISCUSSION

Large and stockily-built salamander species are better able to cope with conditions of drought than small and slender ones (Spight, 1968). For example, the stocky *Salamandra salamandra* may survive dehydration to

TABLE 2. Number of *Chioglossa lusitanica* observed in particular micro-habitats under moist and dry weather conditions. Moist conditions are those of high humidity or rain or both (for definitions see Table 1).

Weather conditions	Microhabitat			
	Hole	Dry-stone wall	Base of tree	Out in the open
<i>Juveniles</i>				
Moist (n=16)	22 (11%)	19 (10%)	140 (73%)	12 (6%)
Dry (n=12)	5 (21%)	9 (38%)	5 (21%)	5 (21%)
<i>Males</i>				
Moist (n=16)	71 (11%)	14 (2%)	514 (82%)	26 (4%)
Dry (n=12)	31 (63%)	3 (6%)	13 (27%)	2 (4%)
<i>Females</i>				
Moist (n=16)	64 (15%)	20 (5%)	322 (76%)	19 (5%)
Dry (n=12)	21 (72%)	3 (10%)	4 (14%)	1 (3%)

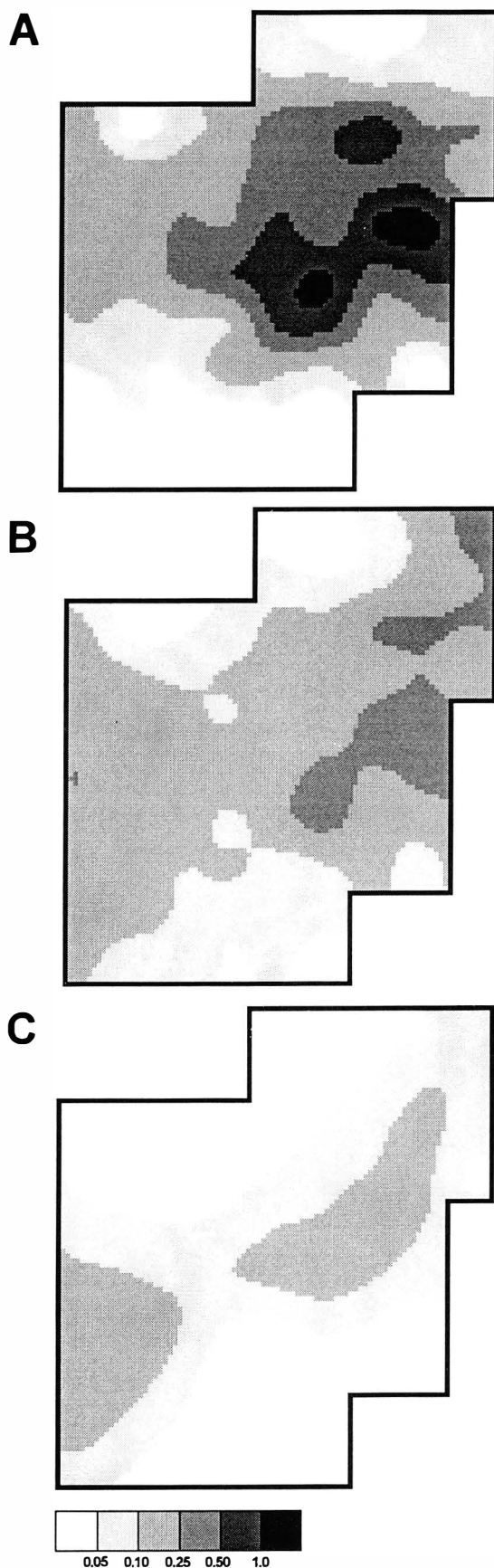


FIG. 3. Spatial models describing the density distribution of male (A), female (B) and juvenile (C) *Chioglossa lusitanica* (for formulae see text). The shading represents the number of salamanders predicted to be found per unit of space and time (grid cells and visits respectively).

the extent of losing 40% of body mass, while slender salamanders are likely to succumb if water loss exceeds 25% (Littleford *et al.*, 1947; Warburg & Degani, 1979). Within a species, the small body size and correspondingly high surface area:volume ratio of juveniles renders them more sensitive to desiccation than adults (Bellis, 1962; Semlitsch, 1981) and gravid females tolerate greater water loss than males of corresponding size (Ray, 1958). In *C. lusitanica* juveniles were observed closer to the water than adults (Arntzen, 1981, 1995; Lima, 1995), and at breeding sites females were found at relatively dry places whereas most males were in or near the water (Arntzen, 1979), thus conforming to expectations from body shape and size. A strong moisture-dependence of *C. lusitanica* is also apparent from an analysis of its geographical range, of which precipitation appears to be the prime determinant (Arntzen, 1981; Teixeira, 1999).

In the present study, the above-ground density of *C. lusitanica* was highest in the vicinity of the stream, either on account of the exit-entry holes (males) or on account of the underground watercourse (juveniles and females). Density was also high near dry stone walls (juveniles and males), in areas with a higher than average density of trees (adults) and in areas of dense undergrowth (females). Interpretation of the results is, however, not entirely straightforward because they simultaneously describe retreat, surface activity and microhabitat. Moreover, the observed distances of salamanders to the holes and to the underground watercourse are correlated. Surface activity increased with high values of relative humidity and rain, and above-ground density decreased with increasing distance from the stream. Dispersion was higher in wet weather conditions than when it was dry. Together with the higher numbers at exit-entry holes in dry weather than in wet weather, the observations indicate that the underground watercourse served as a retreat from which the salamanders would visit the outside. The excursions presumably served for foraging and not for finding sexual partners, because mating (and egg-deposition) does not generally take place above ground, but in secluded places (Arntzen, 1981). The high number of salamanders observed at the bases of trees and in areas of dense vegetation may reflect the availability of food or shelter, or both. The association with dry-stone walls in dry weather conditions (when salamanders tend to be less active) indicates that walls function as retreats rather than as foraging grounds. Similar dependencies were described for *Plethodon cinereus* and *P. richmondi* that were found climbing the vegetation on wet nights, but did not leave their shelters under dry weather conditions (Jaeger, 1971).

The spatial models showed differences in habitat utilization for males, females and juveniles. The observations may well reflect different ecological and behavioural responses by the sexes. The low explanatory power of the spatial model derived for females may be influenced by the fact that we did not distinguish be-

tween gravid and non-gravid individuals. Similarly, the relatively low explanatory power of the spatial model derived for juveniles may be influenced by the classification of different life stages (e.g. newly-metamorphosed, near-adult) as a single group. A particular feature of the juvenile distribution was the high numbers in the stone wall adjacent to the pond. Since it is not uncommon for juvenile salamanders to stay close to their natal site (Palis, 1997; Semlitsch, 1998), the metamorphosis of *C. lusitanica* from the local pond (where larvae have been observed) would help to explain this observation.

Moisture and precipitation have been related to foraging activity in *Mertensiella caucasica* (Tarkhnishvili & Serbinova, 1993), in plethodontids (Hairston, 1949; Heatwole, 1962; Salvidio, 1993; Cimmaruta *et al.*, 1999) and other salamanders (Joly, 1968; Semlitsch, 1981, 1983; Degani & Mendelssohn, 1982; Beneski *et al.*, 1986). Declines in salamander activity and foraging ranges with decreasing precipitation have also been observed in more typical *C. lusitanica* habitat, along fast-flowing mountain streams (Arntzen, 1981, 1995; Lima, 1995). It is unclear, however, whether this decrease is to be related to a change in shelter (closer to the brook) or to change in activity. In our study area, clearly, the shelter was fixed and the activity pattern changed. The similarity in response between *C. lusitanica* and some plethodontid salamanders to changing environmental parameters, the description of similar micro-distributions, and the extreme dependence on moist habitats support the hypothesis that these taxa are subject to evolutionary convergence (Wake & Özeti, 1969; Arntzen, 1994b).

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REFERENCES

- Arntzen, J. W. (1979). De levenswijze van de goudstreepsalamander, *Chioglossa lusitanica*. MSc thesis, University of Amsterdam, Amsterdam.
- Arntzen, J. W. (1981). Ecological observations on *Chioglossa lusitanica* (Caudata, Salamandridae). *Amphibia-Reptilia* **1**, 187-203.
- Arntzen, J. W. (1994a). Speedy salamanders: sedentariness and migrations of *Chioglossa lusitanica*. *Rev. Esp. Herpetol.* **8**, 81-86.
- Arntzen, J. W. (1994b). Allometry and autotomy of the tail in the golden-striped salamander. *Chioglossa lusitanica*. *Amphibia-Reptilia*, **15**, 267-274.
- Arntzen, J. W. (1995). Temporal and spacial distribution of the golden-striped salamander (*Chioglossa lusitanica*) along two mountain brooks in northern Portugal. *Herpetol. J.* **5**, 213-216.
- Bellis, E. D. (1962). The influence of humidity on wood frog activity. *Amer. Midl. Natur.* **68**, 139-148.
- Beneski, J. T. Jr., Zalisko, E. J. & Larsen, Jr. (1986). Demography and migratory patterns of the eastern long-toed salamander, *Ambystoma macrodactylum columbianum*. *Copeia* **1986**, 398-408.
- Cimmaruta, R., Forti, G., Nascetti, G. & Bullini, L. (1999). Spatial distribution and competition in two parapatric sibling species of European plethodontid salamanders. *Ethol. Ecol. Evolut.* **11**, 383-398.
- Degani, G. & Mendelssohn, H. (1982). Seasonal activity of *Salamandra salamandra* (L.) (Amphibia: Urodela: Salamandridae) in the headwaters of the Jordan River. *Israel J. Zool.* **31**, 77-85.
- Faria, M.M., Sequeira, F., Gonçalves, H. & Meneses, C. (1996). Hábitos reprodutivos de salamandra-lusitânica, *Chioglossa lusitanica*, em três minas de Valongo (Norte de Portugal). *IV Congresso Luso-Espanhol de Herpetologia*. Porto.
- Gonçalves, L. (1962). A reprodução de *Chioglossa lusitanica* Bocage. Algumas notas. *Naturalia* **8**, 1-3.
- Goux, L. (1957). Contribution a l'étude écologique, biologique et biogéographique de *Chioglossa lusitanica* Barb. (Urodela, Salamandridae). *Bull. Soc. Zool. Fr.* **82**, 361-377.
- Hairston, N. G. (1949). The local distribution and ecology of the plethodontid salamanders of the southern Appalachians. *Ecol. Monog.* **19**, 47-73.
- Hairston, N. G. (1987). *Community Ecology and Salamander Guilds*. Cambridge University Press. Cambridge.
- Heatwole, H. (1962). Environmental factors influencing local distribution and activity of the salamander *Plethodon cinereus*. *Ecology* **43**, 460-472.
- Jaeger, R. G. (1971). Moisture as a factor influencing the distributions of two species of terrestrial salamanders. *Oecologia* **6**, 191-207.
- Joly, J. (1968). Données écologiques sur la salamandre tachetée *Salamandra salamandra* (L.). *Ann. Sci. Nat. Zool. Biol. Anim.* **10**, 301-366.
- Lima, V. (1995). Estudo comparativo de alguns aspectos da biologia de *Chioglossa lusitanica* em duas populações do noroeste de Portugal. MSc thesis, Faculty of Sciences, University of Porto, Porto.
- Littleford, R. A., Keller, W. F. & Phillips, N. E. (1947). Studies on the vital limits of water loss in the plethodont salamanders. *Ecology* **28**, 440-447.
- Palis, J. G. (1997). Breeding migration of *Ambystoma cingulatum* in Florida. *J. Herpetol.* **31**, 71-78.
- Ray, C. (1958). Vital limits and rates of desiccation in salamanders. *Ecology* **39**, 75-83.
- Salvidio, S. (1993). Life history of the european plethodontid salamander *Speleomantes ambrosii* (Amphibia, Caudata). *Herpetol. J.* **3**, 55-59.

- Semlitsch, R. D. (1981). Terrestrial activity and summer home range of the mole salamander (*Ambystoma talpoideum*). *Can. J. Zool.* **59**, 315-322.
- Semlitsch, R. D. (1983). Structure and dynamics of two breeding populations of the eastern tiger salamander, *Ambystoma tigrinum*. *Copeia* **1983**, 608-616.
- Semlitsch, R. D. (1998). Biological delineation of terrestrial buffer zones for pond-breeding salamanders. *Conserv. Biol.* **12**, 1113-1119.
- Siegel, S. & Castellan Jr., N. J. (1988). *Nonparametric Statistics for the Behavioural Sciences*. Singapore: McGraw Hill.
- Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. 2nd Ed. W. H. Freeman & Company. San Francisco.
- Spight, T. M. (1968). The water economy of salamanders: evaporation water loss. *Physiol. Zool.* **41**, 195-203.
- Tarkhnishvili, D. N. (1994). Interdependences between populational, developmental and morphological features of the Caucasian salamander, *Mertensiella caucasica*. *Mertensiella* **4**, 315-325.
- Tarkhnishvili, D. N. & Serbinova, I. A. (1993). The ecology of the Caucasian salamander (*Mertensiella caucasica* Waga) in a local population. *Asiatic Herpet. Res.* **5**, 147-165.
- Teixeira, J. (1999). Biogeografia de salamandra-lusitânica, *Chioglossa lusitanica*. MSc thesis, Faculty of Sciences, University of Porto, Porto.
- Teixeira, J., Sequeira F., Alexandrino J. & Ferrand N. (1999). Bases para a conservação da salamandra-lusitânica (*Chioglossa lusitanica*) em Portugal. *Estudos de Biologia e Conservação da Natureza*, **22**. ICN.
- Titus, T. A. & Larson, A. (1995). A molecular phylogenetic perspective on the evolutionary radiation of the salamander family Salamandridae. *Syst. Biol.* **44**, 125-151.
- Veith, M., Steinfartz, S., Zardoya, R., Seitz, A. & Meyer, A. (1998). A molecular phylogeny of "true" salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. *J. Zool. Syst. Evol. Res.* **36**, 7-16.
- Vences, M. (1990). Untersuchungen zur Ökologie, Ethologie und geographischen Variation von *Chioglossa lusitanica* Bocage 1864. *Salamandra* **26**, 267-297.
- Vences, M. (1993). Habitat choice of the salamander *Chioglossa lusitanica*: the effects of eucalyptus plantations. *Amphibia-Reptilia* **14**, 201-212.
- Vences, M. (1997). *Chioglossa lusitanica*. In Pleguezuelos, J. M. (ed.). *Distribución y Biogeografía de los Anfíbios y Reptiles en España y Portugal*. Monografías de Herpetología, **3**. Granada.
- Wake, D. B. & Özeti, N. (1969). Evolutionary relationships in the family Salamandridae. *Copeia* **1969**, 124-137.
- Warburg, M. R. & Degani, G. (1979). Evaporative water loss and uptake in juvenile and adult *Salamandra salamandra* (L.) (Amphibia, Urodela). *Comp. Biochem. Physiol.* **62A**, 1071-1075.

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SELECTION OF TADPOLE DEPOSITION SITES BY MALE TRINIDADIAN STREAM FROGS, *MANNOPHRYNE TRINITATIS* (DENDROBATIDAE): AN EXAMPLE OF ANTI-PREDATOR BEHAVIOUR

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Trinidad's only dendrobatid frog, *Mannophryne* (= *Colostethus*) *trinitatis*, lives by the small streams draining the slopes of the Northern Range mountains and at Tamana Hill in the Central Range. Adults are often very abundant, but tadpoles are found patchily in the streams. In the absence of two potential predators – the fish *Rivulus hartii* and shrimps of the genus *Macrobrachium* – tadpoles are abundant in pools. Where the predators are present, tadpoles are uncommon or absent. Tadpoles may also be found in small, isolated bodies of water at some distance from streams. Males carrying tadpoles retained them for 3-4 days, in the absence of suitable pools. When presented with a choice of pools, males preferred to deposit their tadpoles in pools lacking predators. There were differences in behaviour between males from the northern and southern slopes of the Northern Range. For example, north coast males deposited tadpoles in pools containing other conspecific tadpoles in preference to empty pools, whereas males from southern slopes made the opposite choice. When presented only with pools containing predators (i.e. shrimps or fish), north coast males shed their tadpoles in damp leaf litter rather than in the pools, while males from the southern slopes deposited tadpoles in pools with shrimps – predators uncommon in the southern slopes streams. The results indicate that male frogs spend some time searching for predator-free pools in which to deposit their tadpoles. These results are discussed in the context of other examples of anti-predator reproductive behaviour in frogs, and of life history evolution under the influence of different selective pressures.

Key words: Dendrobatids, Trinidad, tadpole-deposition, predator-avoidance, *Rivulus*

INTRODUCTION

A key challenge for ecologists is the identification and measurement of the factors, both biotic and abiotic, that determine the distribution of organisms. The rivers and streams of Trinidad's Northern Range mountains have been a fruitful source of observations and experiments on both proximate factors that affect short term changes and longer term influences that can select for life history and behavioural variables: examples include the vast literature on guppies and their interactions with predators (see Endler, 1995 for review), and work on the non-lethal impacts of piscivorous fish on *Rivulus hartii* dispersal (Fraser *et al* 1995).

Comparatively neglected vertebrate components of the species assemblages of these streams are the tadpoles of several anurans. In the slow-flowing reaches of rivers these include those of *Hyla geographica*, *Hyla boans* and *Bufo marinus*, while in the faster tributaries of the hillsides are those of the stream frog *Mannophryne* (= *Colostethus*) *trinitatis* Garman, Trinidad's only dendrobatid (Murphy, 1997). This report concerns the interaction between *M. trinitatis* and two potential tadpole predators of these streams, the killifish *Rivulus hartii* and shrimps of the genus

Macrobrachium. We deal with the taxonomic problem associated with *M. trinitatis* in the Discussion.

M. trinitatis lives in and around the small mountain streams of Trinidad and the adjacent part of Venezuela (Murphy, 1997; La Marca, 1992). Adults are small (males 25 mm snout-vent length and females 28 mm) and diurnal. Although Sexton (1960) claimed that males were territorial, Wells (1980a) found that it was the females who defended small territories, with the males frequently changing their locations. Males attract females by calling from crevices beneath rocks, turning jet black when they do so. Eggs are laid on land, though deposition has rarely been observed. Praderio & Robinson (1990) found egg clutches on wet leaves or damp soil near streams. Males apparently guard the eggs, but this behaviour has not been formally described (Kenny, 1969; van Meeuwen, 1977). Once the eggs hatch, the tadpoles somehow get on to the male's back, attaching by their oral discs. The male then carries them to water where they complete their development to metamorphosis.

Wells (1980a) suspected that males could carry their tadpoles for several days but that once a 'suitable' pool was found, all tadpoles were deposited within a few hours. Placing a pan of water in a dry stream bed, Wells (1980a) found that eight males deposited tadpoles in the pan within one hour. Males clung to the pan rim, repeatedly dipping their bodies into the water and allowing the tadpoles to drop off.

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In Trinidad, stream frogs are found at two rather different and widely separated locations. In the north, they are abundant alongside higher elevation streams (above the 200 m contour) of the Northern Range mountains. The only other known population lives in and around Tamana cave, part of a limestone hill 21 km to the south and separated from the Northern Range by a low-elevation plain (< 100 m a.s.l.) (Kenny, 1969). Cummins & Swan (1995) reported differences in the reproductive characteristics of Northern Range and Tamana stream frogs, possibly related to differences in predation. The stream running through Tamana cave contains no fish and any tadpole predation is likely to be opportunistic, by predators such as snakes. (Kenny, 1979). The northern and southern watersheds of the Northern Range are biogeographically somewhat distinct, and related to Trinidad's origin as a part of the South American mainland (Kenny, 1995). In the southern watershed, streams where stream frogs are found commonly contain populations of the killifish *Rivulus hartii*. In the northern drainage, *R. hartii* is distributed patchily and the more common potential tadpole predators are shrimps of the genus *Macrobrachium* (Fraser *et al.*, 1995).

Given the abundance of adult and juvenile stream frogs beside the southern Northern Range streams, it is surprising how few tadpoles seem to be present (Cummins & Swan, 1995). The investigation reported here was initiated by the chance finding of a pool in one such stream containing several hundred stream frog tadpoles. Since the number of tadpoles carried by each male ranges from 6 to 13 (Cummins & Swan, 1995), this observation indicated that many males had deposited their tadpoles there. As *Rivulus* were absent from this pool, it is possible that selective anti-predator tadpole deposition was occurring.

We therefore propose that male stream frogs carrying tadpoles actively search for predator-free pools, and selectively deposit their tadpoles in such pools. We report here on a field survey and a laboratory test which together support this hypothesis.

MATERIALS AND METHODS

STUDY SITES

We surveyed five Northern Range streams – three in the southern drainage and two in the northern drainage – and recorded the presence of tadpoles and predators. Surveys were carried out variously during the rainy season (July–August) of 1996 and 1998, and during the dry season (May) of 1997. The southern streams surveyed were:

(1) *Lopinot* (61° 20' W; 10° 41' N). The stream crosses the Lopinot Road 0.5 km before the village. For a few metres above the road, the stream has been canalized, and it passes under the road via a pipe. There is then a sheer drop of 10 m to the stream bed below the road. This stream was examined in July and August 1996 and 1998 (rainy season) and in early May 1997 (dry season).

(2) *Mount Saint Benedict* (61° 24' W; 10° 39.5' N). The stream is accessed via a footpath from the Pastoral Centre car park above the monastery. Surveyed during July and August 1998 (rainy season).

(3) *Maracas Waterfall* (61° 24' W; 10° 44' N). One of several streams crossing the path to the waterfall and recognized by a bamboo 'tap' made to draw water from the stream. Surveyed during July and August 1998 (rainy season).

The northern drainage streams surveyed were:

(1) *East Maracas Bay 1* (61° 24.5' W; 10° 46' N). On the north coast road, 3 km east of Maracas Bay, this stream crosses the road via a tunnel. Surveyed during July and August 1998 (rainy season) and early May 1997 (dry season).

(2) *East Maracas Bay 2* (61° 25' W; 10° 46' N). On the north coast road, 2 km east of Maracas Bay, a very steep stream also crossing the road via a pipe. Surveyed during July and August 1998 (rainy season).

In addition, we surveyed the stream flowing through Tamana cave for tadpoles and predators, in July and August 1996 and 1998.

STREAM SURVEYS

During the rainy season, Northern Range streams rise and fall very quickly according to rainfall. We surveyed streams once water levels had fallen to a 'normal' level. We measured approximate distances between pools, and pool dimensions, with a measuring tape and metre rule: distance measurements were made to assist re-location of pools on subsequent visits when water level changes could alter their appearance considerably. Presence or absence of tadpoles and predators was noted for each pool. In 1996, twelve pools in the Lopinot stream were sampled by means of handnets in an attempt to count all tadpoles and *Rivulus* present.

COLLECTION OF FROGS CARRYING TADPOLES

We used handnets to catch frogs carrying tadpoles, then transferred the frogs to 0.5 litre or two litre polythene tubs equipped with air holes. The tubs were kept damp inside but had no standing water, to reduce the chance that frogs would deposit tadpoles in the tubs. Frogs were then transported by car to the Zoology Department at the University of West Indies – involving a journey time of 30–120 mins, depending on the collection site. Using this method, tadpoles generally stayed on the frogs' backs throughout collection and transportation, and frog survival was high. However, during a period of exceptionally dry weather in August 1998 when the East Maracas Bay 1 stream dried up completely, several died in transit. Transporting frogs in a large tank with a leaf-litter floor eliminated deaths-in-transit.

On arrival at the laboratory, frogs were transferred to large tanks with a damp leaf-litter base, until required for experimentation. Following experiments, frogs and tadpoles were released at their collection sites.

COLLECTION OF POTENTIAL PREDATORS

We caught freshwater shrimps of the genus *Macrobrachium* by handnet in East Maracas Bay stream 1 and kept them in tanks of aerated dechlorinated tap water in the laboratory. *Rivulus hartii* were caught by handnet in the Mount Saint Benedict stream and also maintained in a laboratory tank. Only large specimens of *R. hartii* (50 mm) and *Macrobrachium* (60 mm) were used for predation experiments.

TADPOLE DEPOSITION EXPERIMENTS

Following a pilot study in 1996, experiments designed to test tadpole deposition behaviour were carried out in 1998 in glass tanks in the laboratory, using frogs recently collected in the field. Experiments were conducted on the day of capture, or the morning following capture. We saw no evidence of tadpole deposition during the 'holding' period, but tadpoles were sometimes detached during frog capture, and we had no way of knowing how many tadpoles might have been deposited prior to capture.

The experimental glass tanks were 100 cm x 30 cm x 40 cm, with wooden-framed mosquito-netting lids. The tank bottom was covered to a depth of about 7 cm with damp leaf litter collected in the North Range forests. When frogs were to be tested for preferences between two aquatic deposition sites, two 2-litre polythene tubs were placed at each end of the tank and embedded in the leaf litter so that this came up to the rim of each tub. A rock was placed in each tub, which was then part-filled with dechlorinated tap water so that the rock protruded, providing a perch for the frogs. To test for deposition preference, the following were added to one or both tubs: (1) 20 well grown and free-swimming *M. trinitatis* tadpoles; (2) one *R. hartii*, enclosed in a small plastic cup with a mosquito-netting cover; or (3) one large *Macrobrachium* in a small plastic cup with a mosquito-netting cover. When tadpoles, fish or shrimps were added to a tub, 100 ml of water from their holding tank was also added. The mosquito-netting covers on cups were intended to keep the

predators in place and prevent them from attacking tadpoles, but to make them detectable by the frogs, visually and/or chemically. The cups were fully submerged in the water.

An individual frog carrying tadpoles was released into the central part of each tank and its behaviour was recorded with the aid of a stopwatch, until all tadpoles had been shed. The frog was then removed and the deposition sites of all tadpoles recorded. Predators were given at least 30 minutes to settle before a frog was introduced to the tank. After each individual trial, the plastic tubs were washed and the water was replaced.

RESULTS

DISTRIBUTION OF TADPOLES, *RIVULUS* AND SHRIMPS

Northern Range, rainy season. Tadpoles and predators were found together in only four out of 129 pools. *Rivulus* occurred only in the southern streams and shrimps occurred in one of the northern streams (Table 1).

In the Lopinot stream, *Rivulus* occurred only below the road, suggesting that this acted as a barrier to dispersal. In 1996, we counted the numbers of tadpoles in seven pools above the road (mean 388, range 75-900) and related these to pool size (Spearman's rank correlation: $r_s=0.96$; $P<0.001$).

In 1998, repeat surveys showed that tadpole/predator distributions remained essentially unchanged over several weeks, despite the occurrence of major spates. Our observations were not detailed enough to exclude the possibility of tadpoles being washed downstream, but there was not a noticeable loss of tadpoles from pools.

We occasionally found tadpoles in pools away from streams. On our first visit to the Maracas Waterfall, a pool close to the stream – and formed by the roots of a tree – contained 10 small tadpoles and no *Rivulus*; a week later, it contained *Rivulus* and only three tadpoles. We also found tadpoles at (1) Mount St Benedict, in a water-filled seed-pod, 5 m from the stream; (2) on the summit of El Tucuche (900 m a.s.l.), in several isolated

TABLE 1. Numbers of rainy-season Northern Range pools in different streams containing *M. trinitatis* tadpoles and/or two kinds of potential predators, *Rivulus* and shrimps.

Stream	Tadpoles	<i>Rivulus</i>	Shrimps	Tadpoles and <i>Rivulus</i>	Tadpoles and Shrimps	None
<i>Southern watershed</i>						
Lopinot, 1996	7	4	-	1	-	-
Lopinot, 1998	12	8	-	-	-	13
Mount St Benedict, 1998	-	13	-	-	-	3
Maracas Waterfall, 1998	9	1	-	2	-	3
<i>Northern watershed</i>						
East Maracas Bay 1, 1998	5	-	-	-	-	2
East Maracas Bay 2, 1998	-	-	32	-	1	3
TOTALS	33	26	32	3	1	24

TABLE 2. Tadpole deposition into pools with or without other tadpoles

<i>Southern slopes frogs</i> ¹			
Frog	Number of tadpoles deposited ²		Time ³ (mins)
	Tank with tadpoles	Tank without tadpoles	
1	4	4	74
2	0	7	160
3	0	7	175
4	0	11	125
5	2	5	95
6	0	9	115
7	0	8	126
8	1	4	165
9	0	7	155
Total	7	62	/
Mean±SD	/	/	132±32

<i>North coast frogs</i> ¹			
Frog	Number of tadpoles deposited ²		Time ³ (mins)
	Tank with tadpoles	Tank without tadpoles	
1	4	2	80
2	10	0	25
3	11	0	47
4	6	0	80
5	7	0	95
6	4	0	50
Total	42	2	/
Mean±SD	/	/	63±24

¹Frogs excluded on grounds of abnormal behaviour: southern slopes – one frog, all 7 tadpoles to tank without tadpoles, but in 5 mins. North coast – four frogs: one deposited in leaf litter; one deposited only one tadpole in pool, the rest in leaf litter; one deposited half in the pools; and one all in the pool without tadpoles. Times taken: 7–90 mins. Inclusion of these frogs would not have altered the significance of the results. ²For Southern slopes tadpoles $\chi^2 = 44$, $P < 0.001$; strong preference shown for tank without tadpoles. For north coast tadpoles, $\chi^2 = 36$, $P < 0.001$; strong preference shown for tank with tadpoles. ³A *t*-test on the times taken showed southern slopes frogs took significantly longer ($P < 0.001$) to deposit than north coast frogs.

puddles; and (3) half-way up El Tucuche, in a tree-hole, one metre above the ground.

Northern Range, dry season. In the dry season, Lopinot stream above the road was reduced to one deep cleft containing water and six damp areas with a little water below leaves. The wet areas contained *Rivulus* but no tadpoles. Juvenile and adult frogs were abundant along the stream bed and sides; males were calling, but no males carrying tadpoles were seen. The East Ma-

racas Bay Stream I had visibly flowing water and several shallow pools. Juvenile and adult frogs were abundant, including males carrying tadpoles. Tadpoles were common in the pools and there was no sign of *Rivulus* or shrimps.

Tamana Cave. In the darkest part of the cave, the stream flows through a thick carpet of bat-guano compost. It then flows over a series of worn rocky ledges into the light entrance to the cave. The ledges contain a series of small pools, sometimes undercutting the ledges. It is in these pools, either in darkness or partial light that tadpoles are found. In 1996 and 1998, tadpole numbers were low, with less than 50 in the whole stream in 1996 and fewer than 100 in 1998. No *Rivulus*, shrimp, or any other obvious aquatic predator inhabits this stream, though *Rivulus* has been seen in the stream that flows down the hill, below the level of the cave (C. Cummins, pers. comm.).

TADPOLE DEPOSITION BEHAVIOUR

When a frog carrying tadpoles was introduced to the test tank, it spent some time in exploratory behaviour – moving all around the tank in a series of short jumps, interspersed with variable periods of immobility. When suitable water containers were present, the frog inspected both of these, before eventually depositing his tadpoles in the water.

Once a frog found a suitable pool, it positioned itself on a rock, or on the side of the polythene tub, with its posterior end in the water. It then dipped the hindmost tadpoles in and out of the water every few seconds. These tadpoles then began to wriggle and eventually detached themselves from the frog's back. The frog then moved further into the water to repeat the process for subsequent tadpoles. In about half the cases, all the tadpoles were deposited in a single episode of this behaviour. In all other cases, the frog stopped part way, and moved off to explore the tank again. It then returned to a tub to complete tadpole deposition. In some cases, frogs deposited tadpoles in more than one tub (Table 2). We saw similar behaviour in the field on several occasions.

In the absence of suitable water containers, after exploring the tank the frogs generally remained immobile for long periods. We did not observe them continuously over this period, but simply noted that they eventually deposited all their tadpoles on the moist leaf litter at the bottom of the tank.

Of the 74 frogs tested, 8 (11%) behaved in a manner we judged to be abnormal. These frogs moved rapidly and erratically around the tank and shed their tadpoles very soon after introduction, without the normal exploratory behaviour. Six of these frogs came from the north coast and were collected during the period of drought noted in the Methods section. We interpreted this abnormal behaviour as a sign of stress, and excluded data pertaining to them from the data analysis. They are, however, mentioned as footnotes to the re-

TABLE 3. Tadpole deposition into pools with or without appropriate predator species.

Source of frogs; predator species	Number of frogs tested ¹	Clutch sizes (mean±SD)	Mean % of clutch deposited in pool		Minutes taken (mean±SD) ³
			with predator ²	without predator ²	
Southern slopes; <i>Rivulus</i>	9	7.7±1.8	0	100	229±382
North coast; shrimp	8	5.9±2.3	0	100	89±46

¹Frogs excluded on grounds of abnormal behaviour: southern slopes – one frog which deposited all 5 tadpoles in the pool with the predator within 5 minutes. North coast – two frogs: one deposited all 6 tadpoles in the predator tank within 15 minutes; the other, all 3 to the predator-free pool within 10 minutes. Inclusion of these frogs would not have affected the significance of the results.² In none of the 17 trials showing normal behaviour did a frog deposit any tadpoles into a predator-containing pool. ³A Mann-Whitney *U* test on the times taken showed no significant difference between the two groups: the data were distorted by a single southern slope frog which took 20 hr to deposit its tadpoles, three times longer than any other. Excluding this outlier, southern slopes frogs took 102±38 minutes to deposit, a little longer on average than north coast frogs.

sults tables. We do not think the abnormal behaviour was an effect of handling, since all frogs were introduced to the test tanks without handling, and at least several hours after being collected.

Since we have no way of deciding whether any preference shown is exercised by the adult frog or by the individual tadpoles, we have analysed the results by testing separately the numbers of adults and tadpoles choosing particular tanks. Tadpole numbers were tested using χ^2 , but adult numbers were rarely high enough for this, so we simply quote the numbers found. When adult deposition choices were not clear-cut (e.g. four tadpoles in one tank, two in another) we count their preference according to which tank received the most tadpoles.

Test tank with no pools. Ten frogs – five from the north coast and five from the southern slopes – were observed in a test tank containing leaf litter only. They were observed for short periods three times a day – morning, early afternoon and early evening – until they had shed all their tadpoles on to the leaf litter. The frogs spent much of the time immobile, but did also move around the tank. There was little variability in the results: it took four full days for each of nine frogs to shed all their tadpoles; the remaining frog shed after 3.5 days.

Preference for pools already containing tadpoles. Frogs were given a choice between a pool containing water only and one containing 20 tadpoles.

The results for frogs from the north coast and southern slopes were quite different (Table 2). North coast frogs deposited their tadpoles significantly earlier than southern slopes frogs. Southern slopes tadpoles strongly preferred tanks without other tadpoles; north coast tadpoles displayed the opposite preference. For adults, eight out of nine southern slopes frogs showed a preference for the tanks without tadpoles, while all six north coast frogs showed a preference for the tanks with other tadpoles. During these experiments, we once noticed that large tadpoles attacked tadpoles still on the frog's back while he was depositing them; we also noticed large tadpoles attacking small ones, both in the field and in our experimental tanks (four times in the case of southern tadpoles; once only in northern tadpoles). These attacks sometimes led to small tadpoles being consumed by larger ones.

Avoidance of pools with natural predators. We confirmed that *Rivulus* consume tadpoles in an aquarium environment (e.g. see Cummins & Swan, 1995), and also found that *Macrobrachium* shrimps capture and consume *M. trinitatis* tadpoles.

Frogs carrying tadpoles were given a choice between a pool containing water only and one containing a caged predator. The predators used were either *Rivulus* (southern slopes frogs) or a shrimp (north coast frogs).

In both cases, all frogs deposited all their tadpoles in the predator-free pools (Table 3). This preference was clear-cut in both frogs and tadpoles. The times taken by

TABLE 4. Tadpole deposition into pools with or without the predator species inappropriate to the frog's source.

Source of frogs; predator species	Number of frogs tested ¹	Clutch sizes (mean±SD)	Mean % of clutch deposited in pool		Minutes taken (mean±SD) ³
			with predator ²	without predator ²	
Southern slopes; shrimp	6	6.8±0.8	0	100	117±30
North coast; <i>Rivulus</i>	5	5.9±2.3	0	100	88±29

¹No frogs were excluded from this trial. All frogs tested behaved normally.²In none of the 11 trials did a frog deposit any tadpoles into a predator-containing pool. ³A Mann-Whitney *U* test on the times taken showed no significant difference between the two groups.

TABLE 5. Tadpole deposition into pools where one contains *Rivulus* and the other a shrimp.

Source of frogs	Number of frogs tested ¹	Tadpoles recovered from pools ² (Mean±SD)	Mean (±SD) no. tadpoles deposited in pool		Minutes taken (mean±SD) ³
			with <i>Rivulus</i>	with shrimp	
Southern slopes	7	4.9±1.6	0	4.9±1.6	461±1076
North coast	6	0.7±0.8	0.7±0.8	0	1110±741

¹No frogs were excluded from this trial. All frogs tested behaved normally. ²In both cases, number of tadpoles carried was 5-7 per frog. Tadpoles not recovered from pools were shed on to leaf litter. ³In both cases, mean times taken were greatly affected by single outliers: for southern frogs, one took 2880 minutes, while the next longest was 105 minutes; for north coast frogs, the shortest was 2 minutes and the next shortest 720 minutes. If these outliers are excluded, mean times for southern frogs were 58 minutes and for north coast 1332 minutes, a very substantial difference.

the two groups of frogs were not significantly different, but on average, north coast frogs deposited faster.

Avoidance of pools with unnatural predators. We next tested whether selectivity of deposition is only against the predator normally experienced by these frogs. For this trial, frogs carrying tadpoles were given a choice between a pool containing water only and one containing a caged predator from a different location, i.e. southern slopes frogs were presented with shrimps and north coast frogs with *Rivulus*.

In both cases, all frogs deposited all their tadpoles in the predator-free pools (Table 4). Again, times taken were not significantly different, but – on average – north coast frogs deposited faster.

Preference for pools containing different predators.

We next tested the responses of frogs to two predator environments, one containing *Rivulus* and the other a shrimp. In this case, we have presented the data as numbers of tadpoles deposited, rather than as a percentage of the complete clutch, since many were shed on to leaf litter, rather than into pools.

The results show that southern slopes frogs continued to avoid pools with *Rivulus* but did deposit in pools with shrimp (Table 5). North coast frogs, however, generally avoided both pools, with a few tadpoles deposited in *Rivulus* pools and none in shrimp pools. There was a considerable difference in timing. Southern slopes frogs shed their tadpoles quickly; north coast frogs took a much longer time, with the difference accentuated if two outliers are excluded.

DISCUSSION

In the tiny streams of Trinidad's Northern Range, *M. trinitatis* tadpoles can be found in large numbers in pools that lack two potential predators (*Rivulus hartii* and *Macrobrachium* shrimps). In pools where the predators are found, tadpoles are absent or present in very small numbers.

This distribution pattern has two possible interpretations – either tadpoles are deposited selectively or they are deposited anywhere in streams and only survive where predators are absent. In our view, the very large numbers of tadpoles found in some pools favour selectivity by male frogs.

Male *M. trinitatis* are capable of carrying their tadpoles for several days when no suitable pools are available. When presented with a choice of pools, frogs from the north coast selectively deposited tadpoles in pools containing other tadpoles, rather than in empty pools. On the other hand, frogs from the southern slopes made the opposite selection, depositing preferentially in empty pools.

When presented in the laboratory with the choice of an empty pool or one containing a potential predator (*Rivulus hartii* or *Macrobrachium*), frogs deposited in the empty pools whether the predator originated from the north coast or the southern slopes. When presented with two pools both containing predators, frogs took a much longer time to deposit their tadpoles, many releasing them into the leaf litter rather than the pools. Southern slopes frogs deposited a few tadpoles in pools with shrimps (a predator these frogs should not have experienced); north coast frogs deposited nearly all tadpoles into the leaf litter. Although the number of frogs tested in each experiment was small, the results were clear-cut, with 100% preference shown on many occasions. The number tested was limited by our ability to find and capture frogs carrying tadpoles.

A plausible interpretation of these results is that frogs carrying tadpoles search for pools that are predator-free, and may do this for several days – possibly migrating considerable distances. The number of tadpoles found in a single pool at Lopinot (900) represents the depositions of around 100 frogs. Wells (1980a) found that females – the limiting factor for egg production – occupied territories of 0.6 m² on average but did not describe how far territories extended from the stream. He found 14 females along a stretch of 10 m. A calculation based on the size of the 900-tadpole pool shows a maximum of 35 territories within 2 m of the pool. This suggests that the tadpoles derived from the reproduction of more than the number of frogs in the immediate vicinity.

Magnusson & Hero (1991) showed that predation on eggs has been the main selective force for the evolution of terrestrial oviposition in many neotropical amphibians, including dendrobatids. Our results suggest that predation on hatchlings is an important factor deter-

mining the tadpole deposition behaviour of dendrobatid adults.

SEASONALITY, STREAM FLOW AND TADPOLE/ PREDATOR DISTRIBUTION

The streams of the Northern Range rise and fall very quickly, and in the dry season may cease flowing altogether – being reduced to a few disconnected puddles and damp patches. Furthermore, rainfall is very patchy, with adjacent valleys receiving very different rainfall, especially in the ‘dry’ season when localized showers can occur. Such rainfall patterns cause problems both for the permanent (shrimps, *Rivulus*) and temporary (tadpoles) stream dwellers investigated in this study. Shrimps and *Rivulus* may retreat downstream in the dry season to where water flows all year round, or risk dying as pools dry out. In the heavy rains, all may risk being washed downstream as flow rates and water volumes increase. Our finding that in the late dry season of 1997, *Rivulus* were present in wet puddles of the Lopinot stream above the road, whereas in the wet season of 1998 they were absent from this part of the stream, suggests that localized seasonal extinctions of these fishes can occur.

The distribution of *Rivulus* in these Northern Range streams has been studied extensively (Gilliam *et al.*, 1993; Fraser & Gilliam, 1992; Fraser *et al.*, 1995). These authors have shown that *Rivulus* distribution is determined largely by its interactions with more aggressive piscivorous fish. *Rivulus* is capable of thriving in the lower-level streams, but is driven into higher tributaries by predators such as *Hoplias*.

As has long been known (Jordan, 1923; Seghers, 1978), *Rivulus* possesses considerable jumping ability and is capable of travelling some distance over land: Jordan reports *Rivulus* in pools isolated by at least 20 m from the nearest seasonal stream. However, Fraser *et al.* (1995) noticed that *Rivulus* was absent from some streams where they were expected to occur, and suggested shrimp predation as a cause. An alternative explanation is that stream drying may result in localized extinctions – with recolonization taking some time – despite the jumping powers of *Rivulus*. In the Lopinot stream we studied, a few *Rivulus* were present in dry season (1997) puddles above the road. However, by 1998 we could find no *Rivulus* in that part of the stream during the wet season. In this case, the road may be a major barrier to *Rivulus* recolonization.

The distribution and behaviour of *Macrobrachium* shrimps is less well known. Fraser *et al.* (1995) reported their abundance in the Paria river system, and that they prey on small fish. We found them in one of the north coast streams we surveyed, but not the other. We also found that they too are capable jumpers. In one of our experiments, a shrimp escaped from its ‘cage’ and jumped out of its tub into one containing tadpoles (some of which it consumed).

How well *Rivulus* and tadpoles can maintain their positions during spates requires detailed study, but our

preliminary observations reported here suggest that they are not easily swept downstream. Previous work on the effects of spates on fish (Matthews, 1986; Meffe 1984; Chapman & Kramer, 1991) shows that abiotic factors – i.e. spate severity, stream structure – are important, but also that some species are well adapted to maintaining their positions in such conditions.

PREDATION RISK, CANNIBALISM AND RESOURCES

Our explanation for frog selective deposition behaviour implies that *Rivulus* and shrimp predation are serious problems for *M. trinitatis* tadpoles. In addition, we saw some evidence of tadpole cannibalism. Little is known of the predatory behaviour of the shrimps, but Seghers (1978) showed that *Rivulus* feed mainly on terrestrial insects, by jumping out of the water to catch them. Gut contents from 259 *Rivulus* showed no tadpole remains but some guppies. His sampling period, May to August, coincides with a time when tadpoles should be present, but we suspect that he sampled from lower level streams, where tadpoles do not occur. In any case, if male deposition selectivity is effective, *Rivulus* are likely to encounter tadpoles rarely.

Other tadpole predators do exist. For example, Test *et al.* (1966) report predation on *M. trinitatis* tadpoles (and adults) by the snake *Leimadophis zweifela*. It is also possible that the freshwater crabs that inhabit the streams are amphibian predators, though we are not aware of any reports of this.

Our results showed that north coast frogs chose to deposit tadpoles in pools already containing other tadpoles, whereas southern slopes frogs preferred empty pools. Furthermore, we saw several examples of larger tadpoles attacking small tadpoles as they were being deposited – and later – more frequently by southern than by northern larger tadpoles. Frogs choosing to deposit with other tadpoles may be ‘interpreting’ the presence of tadpoles as evidence of a ‘good’ pond. However, the existence of cannibalism in this species would make this very risky behaviour. Crump (1990) noted that cannibalism provides a source of nutrition in resource-poor environments and Caldwell & de Araujo (1998) found cannibalism commonly among two *Dendrobates* tadpole species deposited in phytotelmata where food scarcity is a common problem. Caldwell & de Araujo did not, however, find any evidence for tadpole deposition selectivity aimed at avoiding cannibalism. More recently, Summers (1999) has reported selective egg and tadpole deposition that reduces cannibalism in *Dendrobates ventrimaculatus*, a species with very small clutch sizes, and inhabiting *Heliconia* leaf axil pools.

Resources available to *M. trinitatis* in Northern Range streams are likely to be severely limited. With low light and nutrient levels, detritus and allochthonous vegetation must be the main resources, and we have seen tadpoles feeding vigorously on damaged mangoes that have fallen into streams. Cannibalism may be a response to these conditions, and selection to avoid pools

containing other tadpoles may be a response to cannibalism.

In addition, we noted several examples in the field where frogs had deposited tadpoles away from a stream in, for example, tree-holes and seed-pods. Wells (1980a) found that frogs quickly deposited tadpoles in a pan of water he placed in a dry stream bed, interpreting this as a response to water availability. However, Cummins & Swan (1995) noted that frogs deposited tadpoles in dishes of water close to a stream – other pools were available, but they contained *Rivulus*. How successful is such extra-stream deposition likely to be? As small pools – such as those formed in seed pods – are likely to be severely lacking in resources, it will be worth investigating under what conditions frogs deposit in such locations.

DURATION OF TADPOLE TRANSPORT

After capture, frogs consistently carried at least some of their tadpoles for four days in the absence of a pool. This consistency is surprising given that we have no way of knowing how long the frogs had been carrying their tadpoles before capture. When presented with suitable pools, frogs generally shed their tadpoles within a few hours, with north coast frogs shedding sooner on average than southern slopes frogs. Cummins & Swan (1995) commented that, in a captive population of *M. trinitatis*, frogs regularly carry tadpoles for 3–4 days even when suitable water is available. This seems at odds with our results – perhaps the captive situation leads to this difference, or the frogs we captured in the field (always from close to streams) had already been transporting their tadpoles for some time.

Wells (1980a,b,c) reported prolonged tadpole transport by *C. inguinalis* (females, up to nine days) and *M. trinitatis* (three to four days). He inferred that tadpoles grew in length during prolonged transport, mainly by utilization of residual yolk, but he also speculated that feeding occurred, his evidence comprising “small amounts of plant detritus” in their guts. We doubt this is the case, as there is no evidence that tadpoles can detach and reattach and the apparent “plant detritus” may well be shed teeth. Downie (1994) found teeth in the guts of non-feeding *Leptodactylus fuscus* tadpoles: without close examination, they could easily be mistaken for plant remains.

Wells suggested that prolonged transport may be advantageous to the tadpoles, their larger size on entering water helping them survive predation. Cummins & Swan (1995), however, noted that prolonged transport may also have costs to both the parent and the tadpoles, – for example in lost feeding opportunities.

Our results suggest that in *M. trinitatis* prolonged transport is mainly associated with the lack of a suitable, predator-free pool. Further work will be needed to establish the costs and benefits of prolonged transport.

PREDATOR AVOIDANCE

Our main finding is that male *M. trinitatis* selectively deposit tadpoles so as to avoid predators. We believe that this is the first time that such evidence has been found from a dendrobatid, though Fandino *et al* (1997) refer to unpublished data showing that *C. subpunctatus* males show some discrimination against pools containing dragonfly nymphs, and Summers' (1999) finding of selective deposition to avoid cannibalism relates to a form of anti-predatory behaviour.

Previous studies have shown that some amphibian species are able to discriminate between pools with fish, pools without fish, and streams as oviposition sites (*Ambystoma barbouri*: Kats & Sih, 1992; *Rana sylvatica*: Hopey & Petranka, 1994; *Rana palustris* and *Bufo americanus*: Holomuzki, 1995; *Hyla chrysoscelis*: Resetarits & Wilbur, 1989) whereas others are not (*Rana temporaria*: Laurila & Teija, 1997). How predators are detected is not known, though Hopey & Petranka (1994) reasoned that chemical detection was more likely than visual detection. In the case of *M. trinitatis*, it is possible that detection is by the larvae and/or the frogs. Our observations on deposition behaviour suggest that the choice is made by the frogs: tadpoles became active and wriggled to detach themselves only after they had been wetted by the dipping behaviour of the frogs. However, it is possible that this behaviour is the result of a cue from the tadpoles. If detection of predators is chemical, it is hard to see how it could be reliable, given variable stream flow rates. It is well established that some amphibian larvae show various forms of predator-detection and anti-predator behaviour (Petranka *et al*, 1987; Lawler, 1989). It has yet to be established whether *M. trinitatis* tadpoles have these abilities.

LIFE HISTORY EVOLUTION IN *C. TRINITATIS*

Cummins & Swan (1995) reported several differences between north coast, southern slopes and Tamana populations of *M. trinitatis* – including adult body size, clutch size and tadpole hatching size. A possible interpretation of the differences was that predation selected for smaller clutches of larger tadpoles. Our data support this comparison, showing differences between the southern slopes and north coast frogs in time taken to deposit tadpoles, and selectivity when presented with two species of predator. The lack of any known tadpole predator of the Tamana population, allied to these findings makes *M. trinitatis* an attractive example for further investigation of the rate of life history evolution. Biogeographically, the freshwater fauna of the north coast is mainly Antillean, but the southern slopes belong more to mainland South America, with the mountains themselves acting as a barrier to the effective mixing of these two populations (Kenny, 1995). Although *M. trinitatis* larvae inhabit streams, this species appears unrestricted by montane barriers.

TAXONOMIC NOTE

The generic name of the Trinidad stream frog has been changed several times, from *Prostherapis* to *Phyllobates* to *Colostethus*. Murphy's (1997) account of Trinidad's amphibians and reptiles follows La Marca (1992) by using the new generic name *Mannophryne*. La Marca erected the genus *Mannophryne* in his catalogue of the Venezuelan frogs on the basis of his own conclusion – in an unpublished MSc thesis – that the genus *Colostethus* is paraphyletic, with the mainly western *collaris* group species, including *trinitatis*, being distinct from the eastern species. La Marca (1994a,b) later substantiated this conclusion and we have followed it here. Species referred to as *Colostethus* in this paper (*subpunctatus*, *inguinalis*) are not members of the *Mannophryne* group.

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REFERENCES

- Caldwell, J. P. & de Araujo, M. C. (1998). Cannibalistic interactions resulting from indiscriminate predatory behaviour in tadpoles of poison frogs (Anura: Dendrobatidae). *Biotropica* **30**, 92-103.
- Chapman, L. J. & Kramer, D. L. (1991). The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia* **87**, 299-308.
- Crump, M. L. (1990). Possible enhancement of growth in tadpoles through cannibalism. *Copeia* **1990**, 560-564.
- Cummins, C. P. & Swan, M. J. S. (1995). Variation in reproductive characteristics of the stream frog *Colostethus trinitatis* on the island of Trinidad. *J. Trop. Ecol.* **11**, 603-618.
- Downie, J. R. (1994). Developmental arrest in *Leptodactylus fuscus* tadpoles (Anura: Leptodactylidae). 1: Descriptive analysis. *Herpetol. J.* **4**, 29-38.
- Endler, J. A. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**, 22-29.
- Fandino, M. C., Lüddecke, H. & Amézquita, A. (1997). Vocalisation and larval transportation of male *Colostethus subpunctatus* (Anura: Dendrobatidae). *Amphibia-Reptilia* **18**, 39-48.
- Fraser, D. F. & Gilliam, J. F. (1992). Nonlethal impacts of predator invasion: facultative suppression of growth and reproduction. *Ecology* **73**, 959-970.
- Fraser, D. F., Gilliam, J. F. & Yip-Hoi, T. (1995). Predation as an agent of population fragmentation in a tropical watershed. *Ecology* **76**, 461-472.
- Gilliam, J. F., Fraser, D. F. & Alkins-Koo, M. (1993). Structure of a tropical stream fish community: a role for biotic interactions. *Ecology* **74**, 1856-1870.
- Holomuzki, J. R. (1995). Oviposition sites and fish-deterrent mechanisms of two stream anurans. *Copeia* **1995**, 607-613.
- Hopey, M. E. & Petranks, J. W. (1994). Restriction of wood frogs to fish-free habitats: how important is adult choice? *Copeia* **1994**, 1023-1025.
- Jordan, D. S. (1923). Habits of the Trinidad guapin, *Rivulus hartii* (Boulenger). *Copeia* **1923**, 69-70.
- Kats, L. B. & Sih, A. (1992). Oviposition site selection and avoidance of fish by streamside salamanders (*Ambystoma barbouri*). *Copeia* **1992**, 468-473.
- Kenny, J. S. (1969). The Amphibia of Trinidad. *Studies of the fauna of Curacao and other Caribbean Islands* **29**, 1-78.
- Kenny, J. S. (1979). Floor plan, environment, and fauna of Tamana cave. *J. Trin. Fld Nat. Club* **1978-79**, 5-9.
- Kenny, J. S. (1995). *Views from the Bridge - a memoir on the freshwater fishes of Trinidad*. Privately published, St Joseph, Trinidad.
- La Marca, E. (1992). *Catálogo taxonómico, biogeográfico y bibliográfico de las ranas de Venezuela*. Universidad de Los Andes, Mérida, Venezuela.
- La Marca, E. (1994a). Taxonomy of the frogs of the genus *Mannophryne* (Amphibia: Anura: Dendrobatidae). *Publs. Soc. Amigos Doñana* **4**, 1-75.
- La Marca, E. (1994b). Biological and systematic synopsis of a genus of frogs from northern mountains of South America (Anura: Dendrobatidae: *Mannophryne*). *Bull. Maryland Herpetol. Soc.* **31**, 40-77.
- Laurila, A. & Teija, A. (1997). Do female frogs choose their breeding habitat to avoid predation on tadpoles? *Oikos* **78**, 585-591.
- Lawler, S. P. (1989). Behavioural responses to predators and predation risk in four species of larval anurans. *Anim. Behav.* **38**, 1039-1047.
- Magnusson, W. E. & Hero, J. M. (1991). Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. *Oecologia* **86**, 310-318.
- Matthews, W. J. (1986). Fish faunal structure in an Ozark stream: stability, persistence and a catastrophic flood. *Copeia* **1986**, 388-397.
- Meffe, G. K. (1984). Effect of abiotic disturbance on coexistence of predator-prey fish species. *Ecology* **65**, 1526-1534.
- Murphy, J. C. (1997). *Amphibians and Reptiles of Trinidad and Tobago*. Krieger Publishing, Malabar, Florida.
- Petranks, J. W., Kats, L. B. & Sih, A. (1987). Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Anim. Behav.* **35**, 420-425.
- Praderio, M. J. & Robinson, M. D. (1990). Reproduction in the toad *Colostethus trinitatis* (Anura: Dendrobatidae) in a northern Venezuelan seasonal environment. *Journal of Tropical Ecology* **6**, 333-341.
- Resetarits, W. J. & Wilbur, H. M. (1989). Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. *Ecology* **70**, 220-228.

- Seghers, B. H. (1978). Feeding behaviour and terrestrial locomotion in the cyprinodontid fish, *Rivulus hartii* (Boulenger). *Verh. Internat. Verein. Limnol.* **20**, 2055- 2059.
- Sexton, O. J. (1960). Some aspects of the behaviour and of the territory of a Dendrobatid frog, *Prostherapis trinitatis*. *Ecology* **41**, 107-115.
- 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.
- Test, F. H., Sexton, O. J. & Heatwole, H. (1966). Reptiles of Rancho Grande and vicinity, Estado Aragua, Venezuela. *Misc. Publs Mus. Zool. Univ. Mich.* **128**, 1-63.
- Van Meeuwen, H. M. (1977). De Trinidadse beekkikker, *Colostethus trinitatis*. *Lacerta* **36**, 3-11.
- Wells, K. D. (1980a). Social behaviour and communication of a dendrobatid frog (*Colostethus trinitatis*). *Herpetologica* **36**, 189-199.
- Wells, K.D. (1980b). Evidence for growth of tadpoles during parental transport in *Colostethus inguinalis*. *J. Herpetol.* **14**, 428-430.
- Wells, K. D. (1980c). Behavioural ecology and social organisation of a Dendrobatid frog (*Colostethus inguinalis*). *Behav. Ecol. & Sociobiol.* **6**, 199-209.

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EFFECTS OF INCUBATION TEMPERATURE ON EMBRYONIC DEVELOPMENT AND SEX DETERMINATION IN THE NORTH AFRICAN AGAMID LIZARD, *AGAMA IMPALEARIS*

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The effects of temperature on incubation time, embryo survival, sex ratio, embryo growth and size at hatching were investigated in the north African Agamid lizard, *Agama impalearis*. Seven constant temperature treatments (spanning 20–36°C) were employed and a split clutch design was used to assign eggs from the same clutch to the different treatments. Incubation time varied significantly with temperature treatments. Embryos incubated at 32°C, 34°C and 36°C hatched between 41 and 46 days, whereas embryos incubated at 26°C and 28°C hatched at 83 and 67 days respectively. Hatching success was higher at 28°C, 30°C, 32°C and 34°C, but much lower at 26°C and 36°C; hatching did not occur at 20°C. Eggs incubated at 26°C and 36°C produced only females. At 28°C, 30°C, 32°C and 34°C, the percentages of males were 9%, 53.5%, 32%, and 58% respectively. These sex ratios can be explained by a temperature-dependent mechanism of sex determination. The relative growth rates are highest early in incubation and lower for several days prior to hatching. The relationship between snout-to-vent length and age of embryos seems to be best described by a polynomial fitted regression. Growth rates at 26°C were much lower than those at 34°C. Constant incubation temperatures affected both snout-to-vent length and body mass at hatching, with maximum body size occurring at intermediate constant incubation temperatures (30°C, 32°C and 28°C). According to this study, the optimal temperatures of embryonic development probably lies within the range 28–34°C. The possible adaptive significance of incubation temperature effects on some life history characteristics of *A. impalearis* is discussed.

Key words: incubation temperature, embryonic development, sex determination, *Agama*

INTRODUCTION

The effects of temperature on embryonic development may constitute an important factor in the ecology and distribution of lizards (Licht & Moberly, 1965). In many species of reptiles, the incubation temperature of eggs can strongly influence a number of developmental traits. In this regard, several works have shown that temperature can induce variation in duration of incubation (Sexton & Marion, 1974; Miller, 1985; Gutzke & Packard, 1987; Van Damme, Bauwens, Braña & Verheyen, 1992), hatching success (Fitch, 1964; Rand, 1972; Venigar, 1973; Sexton & Marion, 1974; Van Damme *et al.*, 1992), sex proportion (Charnier, 1966; Langerwerf, 1983; Janzen & Paukstis, 1991) and growth of embryos (Sexton & Marion, 1974). Incubation temperature may also affect hatchling characteristics (Gutzke & Packard, 1987; Whitehead, Webb & Seymour, 1990; Van Damme *et al.*, 1992; Flores, Tousignant & Crews, 1994; Tousignant & Crews, 1995; Braña & Ji, 2000).

Sexual differentiation has been shown to be sensitive to the incubation temperature during a critical period of embryonic development in many species of reptiles (see Viets, Ewert, Talent & Nelson, 1994; Pieau, 1996 for review). This phenomenon has been termed

temperature-dependent sex determination (Bull, 1980). It was suggested for the first time in an agamid lizard from Africa, *Agama agama* (Charnier, 1966). Subsequent studies showed that TSD is widespread but taxonomically biased in reptiles. Among the species studied so far, this phenomenon has been observed in all crocodylians, most turtles and several lizards (Bull, 1980; Raynaud & Pieau, 1985; Janzen & Paukstis, 1991; Viets *et al.*, 1994). Three TSD patterns have been recognized. *Pattern Ia*: Low temperatures yield 100% (or predominantly) males; high temperatures yield 100% (or predominantly) females. *Pattern Ib*: low temperatures yield 100% (or predominantly) females; high temperatures yield 100% (or predominantly) males. *Pattern II*: low and high temperatures yield 100% (or predominantly) females, with intermediate temperatures yielding various ratios of males. Although TSD was reported in *A. agama* (Charnier, 1966), the sex determining mechanisms in other agamid lizards are still poorly known and only a few species of this group have been studied for their sex-determining pattern as a function of incubation temperatures (Langerwerf, 1983, 1988; Harlow & Shine, 1997, 1999). *A. agama* was reported as having pattern Ib of TSD (high temperatures yield males, low temperatures yield females; Ewert & Nelson, 1991), although only two incubation temperatures were tested (Charnier, 1966). However, studies at warmer temperatures than those previously employed might well

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produce predominantly females in this species (Ewert, Jackson & Nelson, 1994).

It is likely that the microclimatic environment in the natural nest will vary during incubation such that the eggs are not exposed constantly to the optimal temperature for development, but rather to a range of temperatures that encompass this value (Beuchat, 1988). Because it is extremely difficult to locate lizard clutches in the field and consequently to determine the environmental conditions in the natural nesting sites, laboratory studies should provide precise information about the effect of temperature on embryos which can thus be used to establish the physiological limits during development and can help to define abiotic limits imposed on range extension and habitat occupation.

In the present work, using a large range of temperatures and a split clutch design to assign eggs from the same clutch to different treatments, we attempted to determine whether incubation temperature would affect sex determination in the North African agamid lizard, *Agama impalearis* (formerly *Agama bibroni*), a species closely related to *A. agama* (Joger, 1991). We also examined the effects of incubation temperatures on hatching time, embryo growth and survival, and size at hatching in this species.

MATERIALS AND METHODS

During the breeding seasons of 1997 and 1998, 77 gravid females of *A. impalearis* were collected by hand or with a noose in the central Jbilet mountains 25 km north of Marrakech, western Morocco (31°37'N 8°24'W, 580 m above sea level). They were measured for snout-vent length (SVL) and weighed (body mass; BM) to the nearest 0.1 mm and 0.1 g respectively. They were then kept in individual vivaria (70 x 50 x 50 cm) containing a sandy substrate and a pile of flat rocks (shale) that provided refuges for lizards and allowed them to climb. Food (mealworms dusted with commercial vitamin supplement) was offered in excess and water was provided *ad libitum* in small plastic dishes. A 100 W light bulb suspended 15 cm above the vivarium provided heat and light. The vivaria were sprayed with water each morning and lizards were checked for oviposition approximately every hour during the day. Each captive female produced one clutch after which it was returned to the field site.

Eggs freshly deposited were immediately removed and wiped. Post-laying females and their eggs were weighed to the nearest 0.1 g and 0.01 g, respectively. Eggs were scored for possible viability at oviposition and all eggs judged to exhibit reduced viability and to be at risk of hatching failure were not incubated. The eggs were then placed individually in covered plastic cups (150 ml) that contained moistened vermiculite (approximately -200 kPa water potential). Each clutch was distributed across thermostatically controlled incubators (Memmert, $\pm 0.1^\circ\text{C}$) at seven different incubation temperatures (20, 26, 28, 30, 32, 34 and 36°C). The positions of the cups in the incubators were shifted every

three days to minimize the effects of possible thermal stratification within the incubators. The covers of the cups were removed daily and the eggs ventilated by fanning. The vermiculite moisture content was maintained close to the initial vermiculite/water ratio by adding water. Incubators were usually checked for hatchlings about twice a day. The newly-hatched lizards were weighed (BM to the nearest 0.01 g) and measured (SVL, to the nearest 0.1 mm). To determine the sex, hatched lizards were sacrificed and internally examined under a dissecting microscope. Testes were easily distinguishable from ovaries. Moreover, in males hemipenes were present and observable, whereas Müllerian ducts were regressed; in females, hemipenes were regressed whereas Müllerian ducts were present.

During the course of embryonic development at only two constant temperatures (26 and 34°C), sets of three to four eggs were randomly sampled and dissected at time intervals of 5–13 days according to the incubation temperatures. The length of the embryo was measured between the extremity of the head and the base of the tail at the level of the cloaca (to the nearest 0.1 mm).

To assess the sex ratios of hatchlings in nature, specimens were collected in late August ($n=57$) and late November ($n=65$), periods corresponding to the early and late clutches respectively. Young lizards (two months or less of age) presumably representing the late season clutches within the current annual cohort were segregated by their small size (50 mm SVL or less) during field sampling in November (El Mouden, Francillon-Vieillot, Castanet & Znari, 1997; El Mouden, Znari & Brown, 1999).

STATISTICAL ANALYSIS

Egg mass (log-transformed) was analysed as a dependent variable using multiple regression with the following independent variables: oviposition date (the relative laying date of every female was calculated each year using the first day of May as day 1), female SVL, female condition (estimated by the residuals derived from the regression of log [postpartum mass] and log [SVL]), and the relative clutch size (estimated as the residuals from the regression of log [clutch size] and log [SVL]). We estimated temperature effects on length of incubation by one-way ANOVA, and incubation temperature and sex effects on hatchling size (SVL and BM) using two-way ANOVA with initial egg mass entered as a covariate. Chi-square tests were used to test for differences in embryonic survival and sex ratios. For all tests, statistical significance was accepted for $P < 0.05$.

RESULTS

CLUTCH CHARACTERISTICS

Mean clutch size was 13.7 ± 0.27 eggs (range 9–23, $n=77$). Clutch size was positively related to maternal SVL and BM (excluding clutch mass) (SVL: $r=0.61$, $F_{1,71}=41.9$, $P < 0.0001$; BM: $r=0.67$, $F_{1,72}=59.2$, $P < 0.0001$). It increased by approximately one egg per 4 mm of female SVL.

TABLE 1. Effects of incubation temperature on hatching success, duration of incubation, sex ratio and size at hatching in *A. impalearis*. Values for duration of incubation are given as mean \pm 1SD with sample size below. Sex ratios are given as 100x[male/(male+female)] with number of hatchlings examined in parentheses. Values for snout-vent length and body mass are given as mean \pm 1SD with range in parentheses and sample size the same as for sex ratio.

	Temperatures tested ($^{\circ}$ C)						
	20	26	28	30	32	34	36
Eggs incubated (no.)	51	95	116	83	59	43	75
Hatching success (%)	0	46.3	85	83.1	74.6	88.3	41.3
Duration of incubation (days)	-	83.3 \pm 6.3	67.3 \pm 4.1	54.5 \pm 2.9	45.5 \pm 2.4	43.5 \pm 1.3	41.2 \pm 2.2
Number of groups of eggs		9	6	8	8	5	5
Percentage male (male/female)	-	0%(0/44)	9%(9/90)	54%(37/32)	32%(14/30)	58%(22/16)	0%(0/31)
Size at hatching:							
snout-vent length (mm)	-	29.5 \pm 1.57	30.7 \pm 1.69	31.0 \pm 1.43	30.9 \pm 1.48	29.7 \pm 1.45	28.9 \pm 1.96
	-	(26.1-31.6)	(26.5-34.3)	(26.7-33.7)	(27.4-33.1)	(25.0-32.5)	(24.9-32.2)
body mass (g)	-	1.22 \pm 0.19	1.42 \pm 0.18	1.55 \pm 0.20	1.32 \pm 0.16	1.30 \pm 0.22	1.12 \pm 0.17
	-	(0.85-1.57)	(0.69-1.69)	(0.87-1.93)	(0.94-1.59)	(0.6-1.77)	(0.7-1.43)

Clutch mass (in g) was also positively and significantly related to SVL and BM (SVL: $r=0.54$, $F_{1,72}=26.8$, $P=0.0001$; BM: $r=0.60$, $F_{1,71}=37.2$, $P=0.0001$). Initial egg mass varied considerably among clutches of eggs (ANOVA: $F_{67,789}=93.4$, $P=0.001$). A multiple regression analysis indicated that only date of oviposition explained a significant amount of the variation in initial egg mass among clutches (partial regression coefficient; $\text{prc}=-0.002$, $t=3.17$, $P=0.002$). The oviposition date was negatively related to initial egg mass ($r=-0.33$, $F_{(1,70)}=8.5$, $P=0.005$). The examination of embryos at oviposition showed that their development starts before egg-laying; the eggs contained embryos at stage 28 of the developmental table established by El Mouden, Bons, Pieau, Renous, Znari & Boumezzough (2000) for the same species.

INCUBATION TIME AND EMBRYO SURVIVAL

Duration of incubation remained fairly constant within, but varied considerably between, temperature treatments (ANOVA: $F_{5,34}=128.6$, $P=0.0001$). Mean incubation times decrease at increasing temperature with a negative exponential relationship (Table 1). Temperature strongly affected embryo survival ($\chi^2=83.3$, $\text{df}=6$, $P<0.005$) (Table 1). Hatching occurred in eggs kept at all temperatures tested except at 20 $^{\circ}$ C. The low hatching success rates recorded at 26 and 36 $^{\circ}$ C suggested that these temperatures are close to the lethal limits. Indeed, an analysis of embryos that did not hatch showed that the development at 36 $^{\circ}$ C often stopped at early developmental stages, whereas those incubated at 26 $^{\circ}$ C progressed to more advanced developmental stages.

SEX RATIO

The sex-ratio of hatched lizards varied significantly between different constant temperatures of incubation ($\chi^2=48.2$, $\text{df}=5$, $P<0.005$). The 26 and 36 $^{\circ}$ C incubation

temperatures produced only females, whereas the male proportions were 9% at 28 $^{\circ}$ C, 53.5% at 30 $^{\circ}$ C, 32% at 32 $^{\circ}$ C and 58% at 34 $^{\circ}$ C (Table 1). Binomial tests showed that the deviations of the sex ratios from 1:1 were not significant only for egg samples incubated at 30 and 34 $^{\circ}$ C (χ^2 -test; $P>0.05$ in both cases). The examination of the sex ratio within each group of eggs from the same clutch raised at 32 $^{\circ}$ C, revealed the same responses as at 30 and 34 $^{\circ}$ C. Thus, the sex ratio value which deviates significantly from 1:1 at 32 $^{\circ}$ C is probably due to sample variability and can be considered similar to sex ratios at 30 and 34 $^{\circ}$ C. Based on these findings, it appears that the sex of *A. impalearis* is influenced by incubation temperature. We noted that at 26 $^{\circ}$ C all dead embryos were female, so this eliminates an alternative proposal of pre-determined sex with differential mortality. However, at 36 $^{\circ}$ C – as dead embryos were so young and their sexes could not then be identified – we were not able to check this hypothesis. Nevertheless, the examination of groups of eggs from the same clutch incubated at 30, 32 and 34 $^{\circ}$ C revealed an among-family heterogeneity in sex ratio with, at the same temperature, some groups of eggs from the same clutch giving only males while others produced only females.

Under natural conditions, juvenile sex ratios were 75.4 and 36.9% respectively for early and late clutches ($\chi^2=18.2$, $\text{df}=1$, $P<0.05$). The pooled samples provide a global sex ratio of 54.9% which is close to 1:1 (χ^2 -test; $P>0.05$ in both cases). ($\chi^2=1.18$, $\text{df}=1$, $P>0.05$).

GROWTH

During the entire embryonic period at the two incubation temperatures tested, 26 and 34 $^{\circ}$ C, eggs increased in mass by a factor of 2.6 (mass just before hatching divided by mass at oviposition), and in length and width by factors of 1.2 and 1.5 respectively. This emphasizes the importance of water absorption for embryo development.

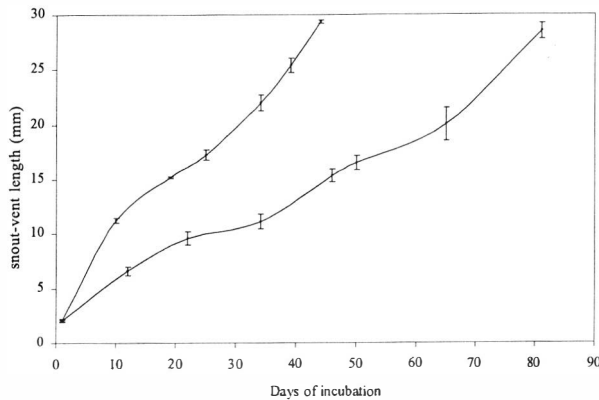


FIG. 1. Snout-vent length of *A. impalearis* embryos incubated at two different constant temperatures (26 and 34°C) as a function of age after oviposition.

The pattern of embryonic growth during incubation at 34 and 26°C in *A. impalearis* is shown in Fig. 1. The relative growth rates (i.e. proportional change in SVL per day) decrease throughout incubation in a similar manner at both temperatures. They are highest early in incubation and decrease thereafter for several days prior to hatching at both temperatures tested (Fig. 2). Therefore, embryonic growth is clearly not exponential. A polynomial fit to the relationship between SVL and age of embryos reveals significant quadratic terms (26°C: $F_{1,21}=482.2$; 34°C: $F_{1,18}=433.1$, $P<0.0001$ in both cases), indicating that growth is unlikely to be linear. Rather, absolute growth is a successively concave and convex downwardly curvilinear relationship and is best described by a polynomial regression. Embryonic growth rates decrease continuously until approximately 15 and 10 days of incubation at 26 and 34°C respectively, and become progressively higher as the body length increases at about 20 days of incubation.

SIZE AT HATCHING

Analysis of variance at each incubation temperature tested revealed that both SVL and BM of hatchlings varied significantly with the average initial individual egg mass of each clutch (one-way ANOVA, $P<0.05$ in all cases), indicating that the two parameters were related to the egg mass at oviposition. Two-way ANOVAs (within the transitional range that produced both males and females), with sex and temperature entered as fac-

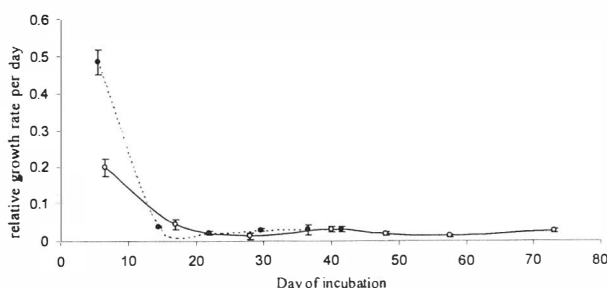


FIG. 2. Relative growth rate of *A. impalearis* embryos during incubation at two different temperatures, 26°C and 34°C.

tors, and initial egg mass as covariate, indicated no significant sex effect or sex \times incubation temperature interaction for either SVL and BM (all $P>0.05$). Thus, more detailed one-way ANOVAs (all the temperatures tested) were used with initial egg mass as a covariate, to test for the incubation temperature effect on both variables. Incubation temperature significantly affected SVL and BM of hatchlings (SVL, $F_{(5,221)}=6.6$; BM, $F_{(5,233)}=13.8$, $P=0.0001$ in both cases). The Duncan's new multiple range test (*a posteriori* test, Kramer, 1956) showed that the eggs incubated at 36, 26 and 34°C produced hatchlings that had significantly lower SVLs and BMs than those incubated at intermediate temperatures (30, 32 and 28°C) (Table 1).

DISCUSSION

The present work provides evidence for significant effects of incubation temperature on several developmental characteristics of *A. impalearis* during the embryonic period, including duration of incubation, embryonic mortality, sex determination, embryonic growth, and hatchling size.

As in other lizards (e.g. Sexton & Marion, 1974; Muth, 1980; Packard & Packard, 1988; Van Damme *et al.*, 1992; Castilla & Swallow, 1996), our data indicate a negative exponential relationship between the length of embryonic development and the incubation temperature. The incubation times in *A. impalearis* are similar to those previously reported by Zbysek (in Rogner, 1997) and Znari, El Mouden & Boumezzough (1998), respectively of 45–54 and 45–58 days at 30°C. The value of 90 days found at 25°C by Santonja (1969) and Santonja & Bons (1972) for the same species is in accordance with the aforementioned trend for incubation duration relative to temperature. Nevertheless, Bons (1964) reported an incubation length varying from 75 to 95 days at 19°C, but the eggs were not placed in a thermostatically controlled incubator. These values are around those we obtained at 26°C. Moreover, all eggs incubated at a constant 20°C failed to develop. The incubation times found in this study are relatively close to those previously cited for an Australian agamid lizard, *Pogona* (= *Amphibolurus*) *barbatus* whose eggs, kept at 25°C hatched in 88–95 days (Bustard, 1966). In contrast, eggs of *A. agama* (Charnier, 1966) took less time to hatch than did those of *A. impalearis* incubated at similar incubation temperatures. On the other hand, in *Iguana iguana*, incubation times at 28°C (Ricklefs & Cullen, 1973) and 30°C (Licht & Moberly, 1965) were much longer at 99 and 73 days respectively. This indicates that the incubation time depends not only on incubation temperature but also on lizard species (e.g. Bustard, 1967; see Packard & Packard, 1988 for review).

The broad thermal tolerance range in *A. impalearis* embryos is comparable to that of certain temperate iguanians (Phrynosomatids) occupying similar habitats in arid areas of North America (Sexton & Marion, 1974; Muth, 1980). By contrast, *I. iguana*, a tropical lizard which normally develops in nests that are well buffered

against temperature changes (Rand, 1972), has a very narrow tolerance range near 30°C (Licht & Moberly, 1965). This suggests adaptation of *A. impalearis* embryos to the variable temperatures likely to be encountered during the natural incubation period which lasts from mid-Spring to mid-Autumn (Znari & El Mouden, 1997). In nature, eggs of *A. impalearis* may hatch between 45 and 60 days (Znari & El Mouden, 1997). This value is close to that determined for eggs maintained at temperatures between 30 and 32°C.

Embryo mortality in *A. impalearis* varied considerably with incubation temperature. This is in accordance with findings in many other reptiles studied from this viewpoint (Sexton & Marion, 1974; Packard & Packard, 1988; Van Damme *et al.*, 1992). Our data indicate that *A. impalearis* eggs hatch over a temperature range with a lower limit between 20 and 26°C and an upper limit exceeding 36°C. The 16-day difference in incubation period between 26 and 28°C is considerably more than the two-day difference between mean values at 34 and 36°C. Similar results were obtained by Sexton & Marion (1974) in an iguanian lizard, *Sceloporus undulatus*, and they interpret this non-linear relationship as indicating that the upper temperature at which incubation was tested is near the thermal maximum for embryonic development.

Genotypic sex determination (GSD) has been reported for *Pogona vitticeps* (Viets *et al.*, 1994) and some other Australian agamids (Harlow & Shine, 1997). In contrast, *Laudakia* (= *Stellio*) *caucasica* (Langerwerf, 1983), *Laudakia* (= *Stellio*) *stellio* (Langerwerf, 1988) and *Chlamydosaurus kingii* (Harlow & Shine, 1999) were reported as having TSD. Two of the three TSD patterns that have been recognized from incubation of reptilian eggs at constant temperatures (Ewert & Nelson, 1991) were reported in agamid lizards (Charnier, 1966; Bull, 1980; Langerwerf, 1983, 1988; see also Viets *et al.* 1994). *L. caucasica* exhibits pattern II (Langerwerf, 1983) while *L. stellio* shows pattern Ib (Langerwerf, 1988). *A. impalearis* exhibits TSD pattern II which is common to the three major reptilian lineages (Ewert & Nelson, 1991). *A. agama*, a species closely related to *A. impalearis*, has been reported to have pattern Ib (Bull, 1980), although only two incubation temperatures were tested (Charnier, 1966). However, as suggested by Ewert *et al.* (1994), studies at warmer temperatures than those previously used might well yield predominantly females in this species as found in *A. impalearis*. In this study, we detected among-family heterogeneity for sex ratio within three incubation temperatures tested. This suggests the existence of genetic sex determination along with the incubation temperature effect, or between-family variability in thermal sensitivity or threshold levels within the ESD system. However, due to insufficient samples we were not able to test for between-family variation and consequently to confirm genetic variation in sex ratio in *A. impalearis*, as done for other reptiles (Rhen & Lang, 1998). The examination of sex ratios among hatchlings from natural populations revealed different values between early and late

clutches with a predominance of males and females respectively. This discrepancy in sex ratio between clutches could be attributed to differences in temperature ranges within nests, the average incubation temperatures being higher for the first clutches (incubated during the summer) than for the second clutches (incubated mostly during autumn).

Several authors have suggested a relationship between the mechanism of sex determination and sexual dimorphism (Charnov & Bull, 1977; Webb & Smith, 1984; Ewert & Nelson, 1991; Ewert *et al.*, 1994). In this regard, Harlow & Shine (1997) investigated the possible adaptation value of TSD by comparing life history characteristics in 20 species of Australian agamids. They found that highly territorial species with extreme sexual dimorphism and male combat are most likely to have TSD, while less territorial species with low or moderate sexual dimorphism do not. Ewert & Nelson (1991) and Ewert *et al.* (1994) pointed out that species with females smaller than males usually have pattern II TSD (pattern-dimorphism congruence hypothesis). In *A. impalearis*, males are very territorial (Bons, 1968, unpublished data) and larger than females (Znari & El Mouden, 1997) which well supports the pattern-dimorphism congruence hypothesis.

Embryonic growth has been investigated in several reptile species (Dmi'el, 1970; Ricklefs & Cullen, 1973; Ackerman, 1980; Renous, Rimblot-Baly, Fretey & Pieau, 1989; Guyot, Pieau & Renous, 1994), but there have been few attempts to model growth data allowing comparisons within or among taxa. Both sigmoidal and exponential growth curves have been observed (Andrews, 1982). Constants obtained by fitting sigmoidal equations to embryonic growth data, as in sea turtles (Ackerman, 1980), are difficult to interpret in lizards including *A. impalearis*, because the size of lizard embryos do not reach plateau levels prior to hatching and asymptotes would therefore be determined arbitrarily (Ricklefs, 1987). Simple exponential equations assume a constant relative growth rate, which is not the case for embryonic growth as was found in *A. impalearis*. To our knowledge, the only lizard species whose embryonic growth has been described mathematically is *I. iguana* (Ricklefs & Cullen, 1973). This species exhibits a growth curve (based on length of embryo) very similar to that observed in *A. impalearis*. However, according to Ricklefs & Cullen (1973) the iguana embryos grew at a constant exponential rate during most of the developmental period. Consequently, the whole growth curve of *A. impalearis*, and probably also that of *I. iguana*, are best fitted to a polynomial model. Although providing good fits to the data, polynomials are arbitrary functions that are difficult to compare across sample taxa since they do not incorporate biologically significant parameters (Ricklefs, 1987).

Embryos of *A. impalearis* grow more rapidly at a high temperature (34°C) than they do at a lower one (26°C). Indeed, developing embryos of numerous lizards complete incubation sooner in warm conditions than they

do in cooler conditions (e.g. Sexton & Marion, 1974; Muth, 1980; Packard & Packard, 1988; Van Damme *et al.*, 1992). This effect of temperature on growth rate in *A. impalearis* embryos is exerted particularly during the early extra-oviductal developmental stages, as also reported in other reptiles (Yntema, 1978; Pieau, 1982). Rapid growth and early completion of development at high temperatures presumably reflect higher rates of metabolism than at lower temperatures (Packard, Tracy & Roth, 1977; Packard & Packard, 1988).

The results from our study indicate that egg mass influences both SVL and BM at hatching. Moreover, the hatchlings from eggs incubated at lower and higher temperatures are on average smaller and lighter than those incubated at intermediate temperatures. Gutzke & Packard (1987) found a similar effect for snout-vent length, but reported a generally decreasing pattern for mass. Packard, Packard & Birchard (1989) did not find significant temperature effects. Burger (1990) considered only two incubation temperatures with increasing size effect. Temperature effects reported by Campos (1993) were evident in length whereas mass remained similar. In ectotherms, with a few exceptions (Atkinson, 1995), the size at a given developmental stage should be reduced by increased rearing temperature (Atkinson, 1994). In our samples, the occurrence of the same effect of incubation temperature on both SVL and BM indicates that the differences in body size are due to actual changes in growth. According to Packard & Packard (1988), the embryos developing at high temperatures may have a higher metabolic rate, but a reduced metabolic efficiency and consequently lower body size. In *Crocodylus johnstoni* hatchlings, residual yolk mass increases positively with incubation temperatures and this pattern of yolk utilization was explained by long incubation periods at low temperatures allowing embryos to metabolize more yolk with a consequent increase in body size (Manolis, Webb & Dempsey, 1987; Deeming & Ferguson, 1989). We did not evaluate residual yolk mass in *A. impalearis* hatchlings but larger sizes at intermediate temperatures suggest a different pattern of yolk utilization – probably with a maximal efficiency occurring at thermal metabolic optima.

An estimation of the optimal temperature of embryonic development for *A. impalearis* reveals that the experimental temperature range of 28–34°C produces the best balance between embryonic survival rate and hatchling size. This optimal thermal range includes the mean temperature recorded in natural nesting sites in early summer (30.4°C) (Znari *et al.*, 1998) and was relatively close to the body temperature selected by adult *A. impalearis* in a thermogradient (TP = $34 \pm 2.3^\circ\text{C}$; Znari & El Mouden, 1998). In addition, the results obtained for the survival rate within the range of incubation temperatures tested showed that the lower and upper thermal limits for embryonic development were much higher and lower respectively than the critical thermal minimum and critical thermal maximum of adults ($\text{CT}_{\text{min}} = 45.6^\circ\text{C}$ and

$\text{CT}_{\text{min}} = 10.7^\circ\text{C}$) (Znari & El Mouden, 1998). These support the multiple optima hypothesis suggested by Huey (1982).

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REFERENCES

- Ackerman, R. A. (1980). Physiological and ecological aspects of gas exchange by sea turtle eggs. *Am. Zool.* **20**, 575-583.
- Andrews, R. M. (1982). Patterns of growth in reptiles. In: *Biology of Reptilia*, Gans C. and Pough, F.H. (Eds). Academic Press, New York.
- Atkinson, D. (1994). Temperature and organism size: a biological law for ectotherms? *Adv. Ecol. Res.* **25**, 1-58.
- Atkinson, D. (1995). Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. *J. Therm. Biol.* **20**, 61-74.
- Beuchat, C. A. (1988). Temperature effects during gestation in a viviparous lizard. *J. Therm. Biol.* **13**, 135-142.
- Bons, J. (1964). Note sur la ponte du lézard *Agama bibroni* (Sauria, Agamidae). *Soc. Sc. Nat. Phys. Maroc* **44** (1 et 2), 65-71.
- Bons, J. (1968). Comportement d'*Agama bibroni* A. Dum. 1851 (Sauria) durant la période de reproduction. *Soc. Sc. Nat. Phys. Maroc* **48** (3 et 4), 93-99.
- Braña, F. & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *J. Exp. Zool.* **286**, 422-433.
- Bull, J. J. (1980). Sex determination in reptiles. *Q. Rev. Biol.* **55**, 3-21.
- Burger, J. (1990). Effects of incubation temperature on behavior of young black racers (*Coluber constrictor*) and Kingsnakes (*Lampropeltis getulus*). *J. Herpetol.* **24**, 158-163.
- Bustard, H. R. (1966). Notes on the eggs, incubation and young of the bearded dragon, *Amphibolurus barbatus* (Cuvier). *Brit. J. Herpetol.* **3**, 252-259.
- Bustard, H. R. (1967). Reproduction in the Australian gekkonid genus *Oedura*, Gray 1842. *Herpetologica* **23**, 176-284.
- Campos, Z. (1993). Effect of habitat on survival of eggs and sex ratio of hatchlings of *Caiman crocodilus yacare* in the Pantanal, Brazil. *J. Herpetol.* **27**, 127-132.

- Castilla, A. M. & Swallow, J. G. (1996). Thermal dependence of incubation duration under a cycling temperature regime in the lizard, *Podarcis hispanica atrata*. *J. Herpetol.* **30** (2), 247-253.
- Charnier, M. (1966). Action de la temperature sur le sex-ratio chez l'embryon d'*Agama agama* (Agamidae, lacertilien). *C. R. Seances Soc. Biol. Fil.* **160**, 620-622.
- Charnov, E. L. & Bull, J. J. (1977). When is sex environmentally determined? *Nature* **266**, 828-830.
- Deeming, D. C. & Ferguson, M. W. J. (1989). The mechanism of temperature dependent sex determination in crocodilians: a hypothesis. *Am. Zool.* **29**, 973-985.
- Dmi'el, R. (1970). Growth and metabolism in snake embryos. *J. Embryol. Exp. Morph.* **23**, 761-772.
- El Mouden, E., Bons, J., Pieau, C., Renous, S., Znari, M. & Boumezzough, A. (2000). Table de développement embryonnaire d'un lézard agamidé, *Agama impalearis* BOETTGER, 1874. *Ann. Sci. Nat. Zool. Paris* **21**, 93-115.
- El Mouden, E., Francillon-Vieillot, H., Castanet, J. & Znari, M. (1997). Age individuel, maturité, croissance et longévité chez l'agamidé nord-africain, *Agama impalearis* Boettger, 1874, étudiés à l'aide de la squeletteochronologie. *Ann. Sci. Nat. Zool. Paris* **18**, 63-70.
- El Mouden, E., Znari, M. & Brown, R. P. (1999). Skeletochronology and mark-recapture assessments of growth in the North African agamid lizard, *Agama impalearis*. *J. Zool. (London)* **249**, 255-261.
- Ewert, M. A. & Nelson, C. E. (1991). Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* **1991**, 50-69.
- Ewert, M. A., Jackson, D. R. & Nelson, C. E. (1994). Patterns of temperature-dependent sex determination in turtles. *J. Exp. Zool.* **270**, 3-15.
- Fitch, V. A. (1964). Temperature tolerance of embryonic *Eumeces*. *Herpetologica* **20**, 184-187.
- Flores, D., Tousignant, A. & Crews, D. (1994). Incubation temperature affects the behavior of adult leopard geckos (*Eublepharis macularius*). *Physiology and Behavior* **55**, 1067-1072.
- Gutzke, W. H. N. & Packard, G. C. (1987). Influence of the hydric and thermal environments on eggs and hatchlings of bull snakes *Pituophis melanoleucus*. *Physiol. Zool.* **60**, 9-17.
- Guyot, G., Pieau, C. & Renous, S. (1994). Développement embryonnaire d'une tortue terrestre, la tortue d'Hermann, *Testudo hermanni* Gmelin, 1789. *Ann. Sc. Nat. Zool., Paris* **15**, 115-137.
- Harlow, P. & Shine, R. (1997). Temperature-dependent sex determination in Australian Agamid lizards. *Abstracts of the Third World Congress of Herpetology*, 2-10 August 1997, Prague, Czech Republic. Zbynek Rocek and Scott Hart, (Eds), p. 91.
- Harlow, P. S. & Shine, R. (1999). Temperature-dependent sex determination in the frillneck lizard, *Chlamydosaurus kingii* (Agamidae). *Herpetologica* **55**, 205-212.
- Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of Reptilia*, 12, 25-91. Gans C. and Pough, F. H. (Eds). Academic Press, London.
- Janzen, F. J. & Paukstis, G. L. (1991). Environmental sex determination in reptiles: ecology, evolution, and experimental design. *Q. Rev. Biol.* **66**, 149-179.
- Joger, U. (1991). A molecular phylogeny of agamid lizards. *Copeia* **1991**, 616-622.
- Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* **12**, 307-310.
- Langerwerf, B. (1983). Über die Haltung und Zucht von *Agama caucasia* (Eichwald, 1831) (Sauria : Agamidae), nebst Bemerkungen zur erfolgreichen Zucht weiterer palaerktischer Echsen. *Salamandra* **19**, 11-20.
- Langerwerf, B. (1988). Management and breeding strategy at the Centro de Investigaciones Herpetologicas in the Canary Islands. *Int. Herpetol. Symp. Captive Propag. Husbandry* **11**, 99-102.
- Licht, P. & Moberly, W. R. (1965). Thermal requirements of embryonic development in the tropical lizard *Iguana iguana*. *Copeia* **1965**, 515-517.
- Manolis, S. C. Webb, G. J. W. & Dempsey, K. E. (1987). Crocodile egg chemistry. In *Wildlife Management: Crocodiles and Alligators*, 445-472. Webb, G. J. W. Manolis, S. C. and Whitehead, P. J. (Eds). Surrey Beatty, Sydney.
- Miller, J. D. (1985). Embryology of marine turtles. In *Biology of Reptilia* 14, 296-328. Gans, C. Billet, F. and Maderson, P. F. A. (Eds). Wiley, New York.
- Muth, A. (1980). Physiological ecology of desert iguana (*Dipsosaurus dorsalis*) eggs: temperature and water relations. *Ecology* **61**, 1335-1343.
- Packard, G. C. & Packard, M. J. (1988). The physiological ecology of reptilian eggs and embryos. In *Biology of The Reptilia*, 16B, 523-606C. Gans C. and Huey, R. B. (Eds). Liss, New York.
- Packard, G. C., Packard, M. J. & Birchard, G. F. (1989). Sexual differentiation and hatching success by painted turtles incubating in different thermal and hydric environments. *Herpetologica* **45**, 385-392.
- Packard, G. C., Tracy, C. R. & Roth, J. J. (1977). The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol. Rev.* **52**, 71-105.
- Pieau, C. (1982). Développement somatique et inversion sexuelle sous l'action de la temperature chez les embryons de reptiles. *Proceedings of First international Colloquium on Pathology of Reptiles and Amphibians*, 29-9/2-10, Angers, France, 233-238. Vago C. and Matz, G. (Eds).
- Pieau, C. (1996). Temperature variation and sex determination in reptiles. *BioEssays* **18**, 19-26.
- Rand, A. S. (1972). The temperature of iguana nests and their relation to incubation optima and to nesting sites and season. *Herpetologica* **28**, 252-253.
- Raynaud, A. & Pieau, C. (1985). Embryonic development of the genital system. In *Biology of Reptilia* 15B, 149-

300. Gans, C. and Bellett, F. (Eds). John Wiley and Sons, New York.
- Renous, S., Rimblot-Baly, F., Fretey, J. & Pieau, C. (1989). Caractéristiques du développement embryonnaire de la Tortue Luth, *Dermochelys coriacea* (Vandelli, 1761). *Ann. Sc. Nat. Zool., Paris* **10**, 197-229.
- Rhen, T. & Lang, J. W. (1998). Among-family variation for environmental sex determination in reptiles. *Evolution* **52**, 1514-1520.
- Ricklefs, R. (1987). Comparative analysis of avian embryonic growth. *J. Exp. Zool. Supplement* **1**, 309-323.
- Ricklefs, R. E. & Cullen, J. (1973). Embryonic growth of the green *Iguana iguana*. *Copeia* **1973**, 296-305.
- Rogner, M. (1997). *Lizards: husbandry and reproduction in the vivarium, geckos, flap footed lizards, agamas, chameleons, and iguanas*. Vol. 1, Krieger publishing company, Malabar, Florida, USA.
- Santonja, J. (1969). *Contribution à l'étude de la denture du lézard Agama bibroni A. Dum.* Thèse Doct. Chir. Dent. Montpellier.
- Santonja, J. & Bons, J. (1972). La différenciation des bourgeons dentaires chez le lézard *Agama bibroni* A. Dum. *Bull. Soc. Nat. Phys. Maroc* **52**, (3 et 4), 227-241.
- Sexton, O. J. & Marion, K. R. (1974). Duration of incubation of *Sceloporus undulatus* eggs at constant temperature. *Physiol. Zool.* **47**, 91-98.
- Tousignant, A. & Crews, D. (1995). Incubation temperature and gonadal sex affect growth and physiology in the leopard gecko (*Eublepharis macularius*), a lizard with temperature-dependent sex determination. *J. Morphol.* **224**, 159-170.
- Van Damme, R., Bauwens, D., Braña, F. & Verheyen, R. F. (1992). Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica* **48**, 220-228.
- Venigar, A. (1973). The effects of temperature on the growth and development of embryos of the Indian python, *Python molurus* (Reptilia: Serpentes: Boidae). *Copeia* **1973**, 171-173.
- Viets, B., Ewert, M. A., Talent, L. G. & Nelson, C. E. (1994). Sex-determining mechanisms in squamate reptiles. *J. Exp. Zool.* **270**, 45-56.
- Webb, G. J. W. & Smith, A. M. A. (1984). Sex ratio and survivorship in the Australian freshwater crocodile *Crocodylus johnstoni*. *Symp. Zool. Soc. Lond.* **52**, 319-355.
- Whitehead, P. J., Webb, G. J. W. & Seymour, R. S. (1990). Effect of incubation temperature on development of *Crocodylus johnstoni* embryos. *Physiol. Zool.* **63**, 949-964.
- Yntema, C. L. (1978). Incubation times for eggs of the turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. *Herpetologica* **34**, 274-277.
- Znari, M. & El Mouden, E. (1997). Sexual dimorphism, reproductive and fat body cycles in Bibron's agama (*Agama impalearis*, Boettger, 1874). *Herpetologica* **53**, 411-422.
- Znari, M. & El Mouden, E. (1998). Variation saisonnière de l'activité et de la thermorégulation chez *Agama impalearis* (Lacertilia, Agamidae) dans les Jbilettes centrales (Maroc). *Bull. Soc. Herp. Fr.* **87-88**, 45-62.
- Znari, M., El Mouden, E. & Boumezzough, A. (1998). Structure et dynamique d'une population d'*Agama impalearis* (Sauria: Agamidae) dans les Jbilettes centrales, Maroc. *Rev. Ecol. (Terre Vie)*, **53**, 293-308.

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FOOD PARTITIONING BETWEEN TWO SYNTOPIC RANID FROGS, *RANA NIGROMACULATA* AND *R. RUGOSA*

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Rana nigromaculata and *R. rugosa* consumed different food resources when they coexisted in Japanese rice fields. *Rana nigromaculata* consumed prey from many taxa including a small proportion of ants (16% in number of the total prey items), while *R. rugosa* ate mainly ants (56%). However, presence of the same terrestrial prey types in the bulk of their diets suggests that the frogs forage on the ground syntopically, and do not partition their feeding sites. Moreover, the comparatively wider mouth of *R. rugosa* (relative to similar sized *R. nigromaculata*), suggests that *R. rugosa* does not eat more ants because of morphological constraints. This dissimilar pattern of food resource utilization seems to have resulted from selection of different prey, as indicated by the stronger avoidance of ants by *R. nigromaculata* compared to *R. rugosa*. This food partitioning may be facilitating their coexistence in rice fields.

Key words: *Rana nigromaculata*, *R. rugosa*, food partitioning, prey selection, syntopic foraging

INTRODUCTION

Rana is a representative genus of the cosmopolitan family Ranidae (Duellman & Trueb, 1986). According to dietary studies from various regions of the world, *Rana* are regarded as generalist predators and are not specialized for eating particular prey taxa (e.g. Houston, 1973; Premo & Atmowidjojo, 1987). Food partitioning of congeneric species of *Rana* has been observed solely between species with larger size differences, such as *R. catesbeiana* and *R. clamitans* (e.g. Werner, Wellborn & McPeck, 1995). In cases of species with similar body sizes, habitats are usually partitioned (e.g. Marshall & Buell, 1955; Loman, 1978). When they are sympatric, they take prey at different sites (e.g. Licht, 1986).

Rana nigromaculata and *R. rugosa* occur sympatrically in some parts of East Asia (Maeda & Matsui, 1999). Fully mature *R. nigromaculata* (SVL > 50 mm) are larger than adult *R. rugosa* (35 ≤ SVL < 60 mm), but the ranges of their body sizes largely overlap when immature individuals are included. The diets of both species have been well studied. *Rana nigromaculata* takes a wide variety of prey taxa, like many other ranids (Hirai & Matsui, 1999), but *R. rugosa* consumes mainly ants (Hirai & Matsui, 2000a). However, the pattern of food partitioning between these species when in sympatry has never been investigated. We therefore examined their food resource utilization in rice fields where they coexist, and further tried to detect factors that caused them to partition food items.

MATERIALS AND METHODS

The study site is located in Iwakura, Kyoto, central Japan, at approximately 35°06'N, 135°52'E and 130 m elevation. *Rana nigromaculata* and *R. rugosa* coexist in

rice fields in this area. Forty censuses were made, these occurring each week from 16 April to 22 October in 1995 and biweekly from 27 April to 24 October in 1996, yielding a total of 34 sampling dates. Besides the two species studied, three other anuran species (*Hyla japonica*, *Rhacophorus arboreus*, and *Rh. schlegelii*) were found at the study site. In contrast to the two ranids, these three species utilized rice fields principally for reproduction and were found only during their breeding periods.

We captured frogs at night between 1800 hr and 0200 hr. Within two hours of capture we anaesthetized frogs in 1% solution of MS-222 and extracted their stomach contents by using forceps. For each frog, we recorded snout-vent length (SVL) and mouth width (MW), and used a toe-clip code for individual identification. After these procedures, we released frogs where they were captured. In the laboratory, we identified stomach contents to the lowest practical taxonomic level, and measured maximum length and width of each prey item. For partially digested prey items, we estimated lengths by measuring width and then using predetermined length-width regressions from intact prey (see Hirai & Matsui, 1999; 2001 for more details).

We classified each prey item as either terrestrial or aquatic on the basis of the habitat in which it typically occurs. Although some prey items were difficult to classify, we regarded maggots (Diptera larvae), adult and larval water beetles (e.g., Dytiscidae), larval caddisflies (Trichoptera), pond-skaters (Gerridae), nymphal dragonflies (Odonata), freshwater shrimps (Amphipoda), and pond snails (e.g. Lymnaeidae) as aquatic prey.

To estimate prey availability, we sampled potential prey invertebrates on the *aze* (slightly elevated narrow trail between adjoining rice fields) by sweep-netting and ground plot techniques on six days (spring: 28 May and 27 June; summer: 25 July and 26 August; autumn: 27 September and 22 October in 1995) (details in method in Hirai & Matsui 1999, 2000a).

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Concurrently with frog collections for diet analyses, we estimated the relative abundance of frogs at the study site. Relative abundance is here defined as the number of individuals collected in one hour by walking along footpaths (the *aze*) between adjoining rice fields. The abundance values were compared by the Wilcoxon signed-rank test.

In order to detect interspecific differences in the use of food resources, we compared frequencies of occurrence of all prey taxa by Fisher's exact probability test, and quantified diet similarity by calculating the simple overlap index, C (Schoener, 1968), where $C_{xy} = 1 - 0.5 \sum |P_{ix} - P_{iy}|$, and P_{ix} and P_{iy} are the proportions of prey taxon i in diets of the two different species, x and y . The values of C range from 0 (no overlap) to 1 (complete overlap). The index was calculated for every two month period (spring: May and June; summer: July and August; autumn: September and October) to evaluate the seasonal change in the feeding relationships of the frogs. The difference in feeding site was tested by comparing frequency of occurrence of aquatic forms using Fisher's exact probability test. The specific difference of prey selection was also examined with Ivlev's electivity index E , (Ivlev, 1961), $E = (P_c - P_a) / (P_c + P_a)$, where P_c and P_a are the proportions of prey items in question in the diet and environment respectively. This index is vulnerable to small proportions in the diet or in the environment (Lechowicz, 1982). We calculated E only for ants, because the proportions of prey taxa other than ants were not large enough for this analysis. The index values vary symmetrically between -1.0 to $+1.0$ as a prey taxon is avoided or preferred respectively. For this analysis, we approximated prey availability within an area of 120 m^2 in each season by combining the total abundances of sweep and ground plot samples.

In addition, we examined relationships between snout-vent length (SVL) and mouth width (MW), between MW and mean prey length, and between MW and maximum prey length. ANCOVA was performed on common logarithms of all these four variables to detect differences between the species. In order to eliminate sampling bias, we included only such frogs that contained at least three prey items in their stomachs to calculate mean and maximum length of prey for each frog. In all diet analyses, stomach contents from recaptured individuals were treated as independent samples because diet composition seems not to differ individually within species.

RESULTS

LIFE HISTORY AND RELATIVE ABUNDANCE

We encountered *Rana nigromaculata* and *R. rugosa* in and around the rice fields throughout the study period. Forty-five out of 389 individuals of *R. nigromaculata*, and 27 of 64 individuals of *R. rugosa* were recaptured more than once (maximum of three times for *R. nigromaculata*, and nine times for *R. rugosa*).

In addition to these two species, we observed reproduction of *Hyla japonica* from early May to mid July ($n=128$), *Rhacophorus arboreus* in mid June ($n=4$), and *Rh. schlegelii* from early May to mid June ($n=33$). Neither rhacophorids had animal prey in their stomachs, but plant pieces or minerals – or both – were found in 21.2% of *Rh. schlegelii* stomachs. From 87.5% of stomachs of *H. japonica*, animal foods were recovered, but this species was collected only in the limited breeding season (Hirai & Matsui, 2000c). Because only the two ranid species utilized rice fields throughout the activity periods, we include only these species in subsequent analyses.

The two species became active at the beginning of May, and were consistently captured until mid-October. During the hibernation periods, from November to April, we could not find any frogs at the study site (Fig. 1). The reproductive season of *R. nigromaculata* was short at the study site, and breeding males were found only in early May. Whilst males were calling from the water fields, other individuals were mostly captured on the ground (*aze*), usually near the water. *Rana rugosa* was found syntopically with *R. nigromaculata*, but breeding males of this species were calling at the water's edge during a prolonged season from late May to late August. We observed that larval *R. nigromaculata* metamorphosed simultaneously at the beginning of July, but larval *R. rugosa* remained in the ditch that does not dry out in summer, and metamorphosed sporadically from September to October.

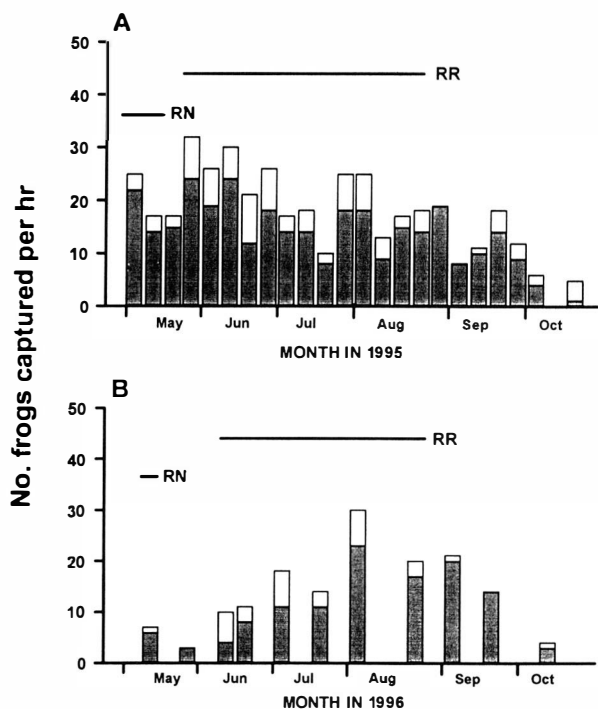


FIG. 1. Seasonal fluctuation in relative abundance of *R. nigromaculata* (RN, hatched rectangle) and *R. rugosa* (RR, open rectangle) in 1995 (A) and 1996 (B). Solid lines indicate periods when males emitting advertisement calls, were captured. No frogs were found in surveys between November and April.

TABLE 1. Diet composition (%) of *Rana nigromaculata* (RN: 2589 prey from 400 frogs, total volume 106145.0 mm³) and *R. rugosa* (RR: 1470 prey from 118 frogs, total volume 13696.6 mm³).

Prey taxon	Frequency of occurrence		Numerical proportion		Volumetric proportion	
	RN	RR	RN	RR	RN	RR
Insecta						
Hymenoptera						
Formicidae	39.5	84.8	16.0	56.3	2.1	9.1
non-Formicid	9.3	12.7	1.9	1.2	1.6	0.9
larvae	5.0	0.9	1.5	0.1	1.1	0.5
Coleoptera	45.3	61.0	11.1	12.7	24.8	30.0
larvae	9.5	8.5	2.9	1.0	3.5	3.8
Diptera	40.0	40.7	12.2	5.0	2.9	3.3
larvae	10.5	11.0	3.6	5.7	1.8	2.3
Lepidoptera	13.8	4.2	2.6	0.4	2.6	0.4
larvae	25.3	14.4	5.7	1.4	14.6	6.8
Trichoptera	0.5	0.9	0.1	0.1	<0.1	<0.1
larvae	0.5	-	0.1	-	<0.1	-
Neuroptera	1.8	-	0.3	-	0.1	-
larvae	0.3	-	<0.1	-	<0.1	-
Hemiptera	31.8	26.3	8.3	3.2	10.7	5.8
Dermaptera	6.5	6.8	1.2	0.6	1.0	1.9
Orthoptera	19.0	5.9	3.6	0.5	9.0	5.4
Odonata larvae	1.0	1.7	0.2	0.1	0.6	1.7
Ephemeroptera	0.3	-	0.1	-	<0.1	-
Thysanoptera	0.3	-	<0.1	-	<0.1	-
Collembola	2.8	3.4	0.5	1.4	<0.1	<0.1
Arachnida						
Araneae	46.5	41.5	17.2	6.0	2.9	4.3
Opiliones	0.5	0.9	0.1	0.1	<0.1	<0.1
Acarina	2.0	7.6	0.3	1.0	<0.1	0.1
Crustacea						
Isopoda	9.0	7.6	2.3	1.4	1.8	4.5
Decapoda	0.5	0.9	0.1	0.1	0.5	4.3
Amphipoda	0.3	-	<0.1	-	<0.1	-
Chilopoda	3.8	2.5	0.6	0.2	0.5	<0.1
Diplopoda	5.3	5.1	1.0	0.4	0.3	0.7
Gastropoda	16.3	3.4	5.1	0.7	2.8	0.5
Oligochaeta	8.0	4.2	1.5	0.3	14.0	13.5
Amphibia						
Anura	0.3	-	<0.1	-	0.9	-

Rana nigromaculata was consistently more abundant than *R. rugosa* in 32 out of 34 censuses (94.1%). Wilcoxon signed-rank test revealed that the relative abundance differed significantly ($P<0.01$) in both 1995 (mean \pm SD, 14.0 \pm 5.9 for 323 *R. nigromaculata*; 4.0 \pm 2.6 for 93 *R. rugosa*) and 1996 (10.9 \pm 6.9 for 120 *R. nigromaculata*; 2.9 \pm 2.7 for 32 *R. rugosa*).

FOOD PARTITIONING

Diet composition. Stomach contents were found in 400 out of a total 443 stomachs of *R. nigromaculata*

(90.3%), and in 118 out of a total 125 stomachs of *R. rugosa* (94.4%). The two species had a wide variety of prey taxa in common (Table 1), but were markedly different in their utilization of some prey taxa. *Rana rugosa* consumed small prey such as ants, mites and small beetles more frequently than did *R. nigromaculata*, and the frequencies of occurrence significantly differed between the two species ($P<0.01$). In particular, ants were ingested by 84.8% of *R. rugosa*, as compared with only 39.3% of *R. nigromaculata*. Numerical proportions of ants also differed markedly

TABLE 2. Comparison of mean number (\pm SD) of prey items found in a stomach of the two species in three seasons. Range and sample size of stomachs are shown in parenthesis.

	Spring	Summer	Autumn
<i>R. nigromaculata</i>	5.0 \pm 3.2 (1-16, 136)	8.0 \pm 7.4 (1-59, 184)	5.4 \pm 4.4 (1-19, 80)
<i>R. rugosa</i>	12.2 \pm 14.3 (1-65, 52)	11.9 \pm 9.1 (2-47, 50)	15.1 \pm 14.4 (2-50, 16)
U-test	$P<0.01$	$P<0.01$	$P<0.01$

between *R. rugosa* (56.3%) and *R. nigromaculata* (16.0%). Instead, *R. nigromaculata* more frequently consumed large prey such as moths (lepidopterans), caterpillars (larval lepidopterans), grasshoppers (orthopterans), and gastropods ($P<0.05$ for caterpillars, $P<0.01$ for the others). By volume, beetles made up the largest proportions in both species, followed by caterpillars, earthworms, and bugs in *R. nigromaculata*, and earthworms in *R. rugosa*.

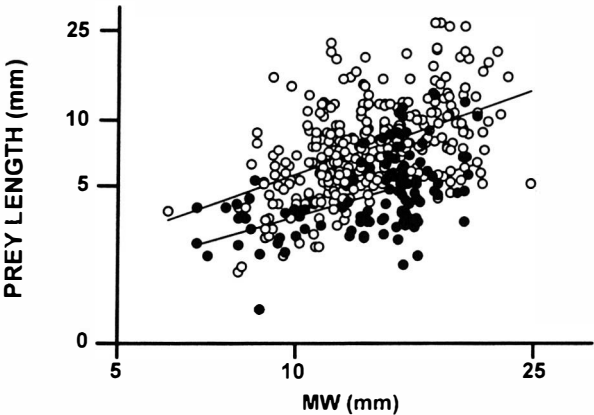


FIG. 2. Relationships between mouth width and mean prey size of *R. nigromaculata* (open circle) and *R. rugosa* (dark circle).

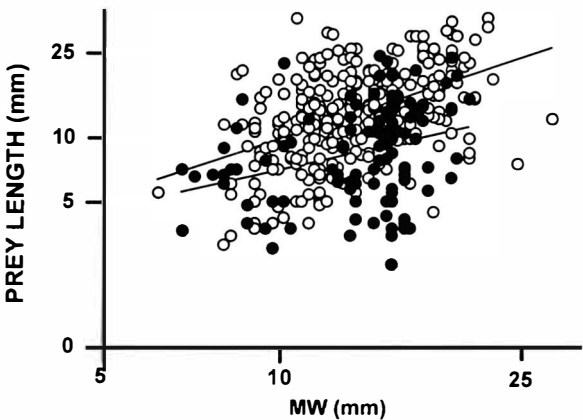


FIG. 3. Relationships between mouth width and maximum prey size of *R. nigromaculata* (open circle) and *R. rugosa* (dark circle).

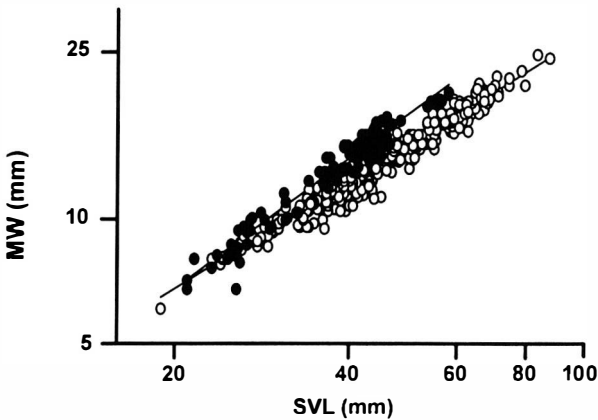


FIG. 4. Relationships between SVL and mouth width of *R. nigromaculata* (open circle) and *R. rugosa* (dark circle).

Seasonal changes and prey selection. *Rana nigromaculata* took significantly fewer prey than did *R. rugosa*, consistently from spring to autumn (Table 2). No particular prey taxon predominated in the diet of *R. nigromaculata*, and various prey such as ants, beetles, dipterans, bugs, spiders were taken in similar proportions. Ants represented only 7.9-21.1% of total prey items in this species. On the contrary, ants were dominant in all seasons in the diet of *R. rugosa* (37.8%-62.9%; Table 3).

Diet similarities showed little seasonal variation, with low values from 0.49 to 0.55. Aquatic forms made smaller contributions numerically than terrestrial ones in the diets of both species (numerical proportions in spring, summer and autumn were 8.5%, 13.6% and 8.0% in *R. nigromaculata*, and 6.8%, 3.9% and 17.0% in *R. rugosa*, respectively). *Rana nigromaculata* took aquatic forms more often in the summer (frequencies of occurrence in spring, summer and autumn were 21.3%, 45.7% and 18.8%, respectively), but *R. rugosa* took them consistently in all seasons (21.2%, 20.0%, and 25.0%, respectively). A significant difference between the species in this frequency was detected only in summer ($P<0.01$).

In the habitat studied, ants were the most easily available prey throughout the seasons (spring, 82.6%; summer, 73.0%; autumn, 69.0%) (Table 4). The electivity indices indicated that both frog species avoided ants, but *R. nigromaculata* avoided them more strongly ($E=-0.82, -0.55, -0.70$ in spring, summer, and autumn, respectively) than *R. rugosa* ($-0.19, -0.07, -0.29$).

Frog and prey sizes. *Rana nigromaculata* (mean \pm SD, 46.7 \pm 12.9 mm; range, 18.9-86.9 mm) was significantly larger in SVL than *R. rugosa* (39.9 \pm 8.4 mm, 20.9-59.7 mm). However, the ranges overlapped considerably. Prey size was significantly correlated with frog size in both species (*R. nigromaculata*: $r_{\text{mean}}=0.48, r_{\text{max}}=0.46$, *R. rugosa*: $r_{\text{mean}}=0.48, r_{\text{max}}=0.30$; $P<0.01$ for all; Figs. 2, 3). Mouth width relative to the body size was narrower in *R. nigromaculata* than *R. rugosa* (Fig. 4), and the relationship between SVL and mouth width differed significantly between the species

TABLE 3. Feeding relationships between *Rana nigromaculata* (RN) and *R. rugosa* (RR). Values are numerical proportions (%) of total prey items. See text for dietary overlap, *C*.

Prey taxon	Spring		Summer		Autumn	
	RN	RR	RN	RR	RN	RR
Insecta						
Hymenoptera						
Formicidae	7.9	57.1	21.1	62.9	11.5	37.8
non-formicid	2.3	0.5	1.0	1.9	4.1	1.7
larvae	3.1	0.2	1.1	-	0.5	-
Coleoptera	16.1	16.2	9.7	11.5	8.0	6.6
larvae	1.2	1.3	3.7	0.8	2.8	0.4
Diptera	24.8	6.5	7.4	4.2	8.9	3.3
larvae	1.9	4.1	4.1	2.7	4.4	17.0
Lepidoptera	1.6	0.2	2.9	0.5	3.0	0.8
larvae	2.6	0.8	6.2	1.7	8.7	2.1
Trichoptera	-	-	0.1	-	-	0.8
larvae	-	-	0.1	-	0.2	-
Neuroptera	0.6	-	0.2	-	0.2	-
larvae	-	-	-	-	0.2	-
Hemiptera	5.0	3.5	7.2	2.5	17.0	4.1
Dermaptera	1.2	0.8	1.2	0.5	1.4	0.4
Orthoptera	3.2	0.2	3.8	0.5	3.9	1.2
Plecoptera	-	0.2	-	-	-	0.4
Odonata larvae	0.2	-	0.3	0.3	-	-
Ephemeroptera	-	-	0.1	-	-	-
Thysanoptera	0.2	-	-	-	-	-
Collembola	0.3	0.3	0.3	-	1.6	7.9
Arachnida						
Araneae	14.2	4.6	19.4	6.2	14.7	9.1
Opiliones	0.3	-	-	0.2	-	-
Acarina	0.3	0.3	0.1	-	0.9	5.4
Crustacea						
Isopoda	3.8	1.1	0.9	2.2	4.8	0.4
Decapoda	-	-	0.1	0.2	-	-
Amphipoda	-	-	0.1	-	-	-
Chilopoda	1.2	0.2	0.4	0.3	0.2	-
Diplopoda	2.0	0.5	0.5	0.3	0.1	0.4
Gastropoda	4.5	1.4	6.5	0.2	1.4	-
Oligochaeta	1.8	0.5	1.6	0.3	0.5	-
Amphibia						
Anura	-	-	-	-	0.2	-
Dietary overlap, <i>C</i>	0.49		0.55		0.51	

(ANCOVA; slopes: $F=55.82$, elevations: $F=415.91$, $P<0.01$ for both). However, *R. nigromaculata* took significantly larger prey than the relatively wider-mouthed *R. rugosa* (slopes: $F_{\text{mean}}=1.09$, $F_{\text{max}}=2.35$, $P>0.05$ for both; elevations: $F_{\text{mean}}=119.18$, $F_{\text{max}}=68.55$, $P<0.01$ for both; Figs. 2, 3).

DISCUSSION

Rana nigromaculata and *R. rugosa* had a wide variety of prey taxa in common, but markedly differed in ant utilization. Dissimilarities in their diets, as also indicated by low overlap values (0.49-0.55), were recognized consistently from spring to autumn. The

TABLE 4. Prey availability estimated from the number of potential prey animals collected by sweep (S) and ground plot sampling (G). See text for the details about availability (Avail.).

Prey taxon	May		Jun		Spring		Jul		Aug		Summer		Sep		Oct		Autumn	
	S	G	S	G	Avail.	%	S	G	S	G	Avail.	%	S	G	S	G	Avail.	%
Formicidae	20	49	3	83	15863	82.6	3	43	36	13	6759	73.0	39	33	14	18	6173	69.0
non-formicid	5	-	4	-	9	<0.1	1	-	28	-	29	0.3	19	-	23	-	42	0.5
larvae	2	-	-	-	2	0.1	-	-	-	-	-	-	3	-	-	-	3	<0.1
Coleoptera	17	-	8	3	385	2.0	7	-	66	-	73	0.8	14	-	8	-	22	0.3
larvae	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	121	1.4
Diptera	66	-	13	-	79	0.4	7	-	164	-	171	1.9	126	-	64	-	190	2.1
Lepidoptera	1	-	-	-	1	<0.1	-	-	6	-	6	<0.1	5	-	3	-	8	<0.1
larvae	-	-	-	-	-	-	-	-	5	1	125	1.4	8	-	3	2	251	2.8
Hemiptera	5	-	2	-	7	<0.1	2	2	108	-	350	3.8	81	-	18	-	99	1.1
Dermaptera	-	-	-	1	120	0.6	-	2	-	6	960	10.4	-	1	-	-	120	1.3
Orthoptera	20	-	45	3	425	2.2	36	1	98	-	254	2.7	68	-	20	-	88	1.0
Thysanoptera	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	3	<0.1
Araneae	31	10	-	3	1593	8.3	-	-	47	3	527	5.7	106	6	35	6	1581	17.7
Isopoda	-	-	-	3	360	1.9	-	-	-	-	-	-	-	-	-	-	-	-
Diplopoda	2	2	-	1	362	1.9	-	-	-	-	-	-	-	-	-	1	120	1.3
Oligochaeta	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	120	1.3

overall dissimilarities result from the difference in the consumption of ants.

Diets of other populations of these two species have been studied in detail (Hirai & Matsui, 1999, 2000a). In these studies, *R. nigromaculata* ate a broad range of prey taxa in similar proportions, and ants represented only 20.3% of total prey items (Hirai & Matsui, 1999). In contrast, *R. rugosa* took numerous ants regardless of differences in body size or habitats, and ants represented 56.8-59.4% of the diet (Hirai & Matsui, 2000a). Thus, the results obtained in this study (16.0% in *R. nigromaculata* and 56.3% in *R. rugosa*) were similar to those of previous studies, and the consistency in the values for *R. rugosa* is clear.

In resource partitioning studies, it is more important to attempt to reveal factors regulating the patterns of resource partitioning than to merely document differences in resource use (Toft, 1985). According to resource partitioning studies in *Rana* (e.g., Marshall & Buell, 1955; Loman, 1978, 1979; Licht, 1986), habitats were partitioned most frequently between congeneric species pairs. Even when congeneric species are in sympatry, they generally take prey from different sites. For instance, *R. pretiosa* forages principally in the water, but *R. aurora* takes prey on the ground (Licht, 1986).

In this study, the terrestrial areas available for foraging were limited to the levee banks between the rice paddies, and there was no significant difference between the species in the proportions of aquatic prey in the diet. Both species eat mainly terrestrial prey. These findings suggest that they take prey on the ground syntopically, and dissimilarities in the diets are not attributable to differences in feeding sites.

Frogs are often gape-limited predators, and the consumable size of prey is determined by the size of gape

or body (Kramek, 1972; Toft, 1980). Narrow-mouthed anurans like dendrobatids and microhylids are specialized for eating small prey such as ants (Toft, 1980; Hirai & Matsui, 2000b). However, in our study, the relatively wider-mouthed *R. rugosa* consumed more ants than the narrower-mouthed *R. nigromaculata*. This result suggests that ant eating by *R. rugosa* is not due to gape-limitation.

These lines of evidence indicate that food partitioning between *R. nigromaculata* and *R. rugosa* is not caused by differences in either feeding site or feeding apparatus, but by different prey selection. In fact, the electivity index suggested that both frogs avoided ants – notwithstanding their abundance in the habitat – but the degree of avoidance was much stronger in *Rana nigromaculata* (*E* ranging from -0.82 to -0.55) than in *R. rugosa* (*E* ranging from -0.29 to -0.07). From these results, we consider that different prey selection is responsible for food partitioning between *R. nigromaculata* and *R. rugosa*, that are congeneric and syntopic.

Food partitioning among coexisting ranids has been explained previously as a result of differences in body size or feeding site (e.g., Stewart & Sandison, 1972; Licht, 1986; Werner *et al.*, 1995). To our knowledge, this study is the first to report food partitioning by differences in prey selection in *Rana*. Such partitioning might be one of the important factors regulating the structure of anuran assemblages in rice fields.

REFERENCES

- Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians*. New York: McGraw-Hill Book Co.
- Hirai, T. & Matsui, M. (1999). Feeding habits of the pond frog, *Rana nigromaculata*, inhabiting rice fields in Kyoto, Japan. *Copeia* 1999, 940-947.

- Hirai, T. & Matsui, M. (2000a). Myrmecophagy in a ranid frog *Rana rugosa*: Specialization or weak avoidance to ant eating? *Zool. Sci.* **17**, 459-466.
- Hirai, T. & Matsui, M. (2000b). Ant specialization in diet of narrow-mouthed toad, *Microhyla ornata*, from Amamioshima island of the Ryukyu archipelago. *Current Herpetol.* **19**, 27-34.
- Hirai, T. & Matsui, M. (2000c). Feeding habits of the Japanese tree frog, *Hyla japonica*, in the reproductive season. *Zool. Sci.* **17**, 977-982.
- Hirai, T. & Matsui, M. (2001). Attempts to estimate the original size of partly digested prey recovered from stomachs of Japanese anurans. *Herpetol. Rev.* **32**, 14-16.
- Houston, W. W. K. (1973). The food of the common frog, *Rana temporaria*, on high moorland in northern England. *J. Zool. London* **171**, 153-165.
- Ivlev, V. S. (1961). *Experimental Ecology of the Feeding of Fishes*. Connecticut: Yale University Press.
- Kramek, W. C. (1972). Food of the frog *Rana septentrionalis* in New York. *Copeia* **1972**, 390-392.
- Lechowicz, M. J. (1982). The sampling characteristics of electivity indices. *Oecologia* **52**, 22-30.
- Licht, L. E. (1986). Food and feeding behavior of sympatric red-legged frogs, *Rana aurora*, and spotted frogs, *Rana pretiosa*, in southwestern British Columbia. *Can. Field-Nat.* **100**, 22-31.
- Loman, J. (1978). Macro- and microhabitat distribution in *Rana arvalis* and *R. temporaria* (Amphibia, Anura, Ranidae) during summer. *J. Herpetol.* **12**, 29-33.
- Loman, J. (1979). Food, feeding rates and prey size selection in juvenile and adult frogs, *Rana arvalis* Niss. and *R. temporaria* L. *Ekol. Polska* **27**, 581-601.
- Maeda, N. & Matsui, M. (1999). *Frogs and Toads of Japan*. Rev. Ed. Tokyo: Bun-ichi Sogo Shuppan. (in Japanese with English abstract)
- Marshall, W. H. & Buell, M. F. (1955). A study of the occurrence of amphibians in relation to a bog succession, Itasca State Park, Minnesota. *Ecology* **36**, 381-387.
- Premo, D. B. & Atomowidjojo, A. H. (1987). Dietary patterns of the crab eating frog *Rana cancrivora* in west Java. *Herpetologica* **43**, 1-6.
- Schoener, T. W. (1968). The *Anolis* lizards of Bimini: Resource partitioning in a complex fauna. *Ecology* **49**, 704-726.
- Stewart, M. M. & Sandison, P. (1972). Comparative food habits of sympatric mink frogs, bullfrogs, and green frogs. *J. Herpetol.* **6**, 241-244.
- Toft, C. A. (1980). Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. *Oecologia* **45**, 131-141.
- Toft, C. A. (1985). Resource partitioning in amphibians and reptiles. *Copeia* **1985**, 1-21.
- Werner, E. E., Wellborn, G. A. & McPeck, M. A. (1995). Diet composition in postmetamorphic bullfrogs and green frogs: Implications for interspecific predation and competition. *J. Herpetol.* **29**, 600-607.

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SHORT NOTES

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FIELD OBSERVATIONS OF ANTI-PREDATOR BEHAVIOURS IN THREE SPECIES OF NEWT (GENUS: *TRITURUS*)

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Key words: newt behaviour, anti-predator, *Triturus*

Newts and salamanders react to potential predators by displaying their aposematic colour patterns, and releasing toxic skin secretions whilst maintaining a rigid posture (Hinsche, 1926; Griffiths, 1996). This response forms an effective protection against predators (Brodie, Nowak & Harvey, 1979; Brodie, Nussbaum & Digiovanni, 1984). However, the posture is highly variable, especially within the family Salamandridae (e.g. Brodie, 1977).

From 1988 to 1995, a study of amphibian population dynamics and migrations was carried out in an area south-west of Bonn [Northrhine-Westfalia, Germany] (Kneitz, 1998; Kupfer & Kneitz, 2000). Amphibians migrating within this area were sampled by perimeter drift-fences and pitfall traps. During the daily censuses, some smooth newts (*Triturus vulgaris*), alpine newts (*T. alpestris*) and crested newts (*T. cristatus*) were

found to display a particular posture, which was identified as anti-predator behaviour (Fig. 1).

The anti-predator behaviour could be induced by touching the newts with a dip-net, but some newts displayed it without any visible stimulus. During spring migrations the behaviour was observed only in terrestrial adults, but – while leaving the pond in August – five juvenile smooth newts, two juvenile alpine newts and one juvenile crested newt (Fig. 1c) expressed it as well. About 1% of the smooth and alpine newts caught in the traps showed anti-predator responses during the spring migration. In crested newts, the frequency was 12% in males and 7% in females (Table 1).

Great variation in the intensity of the posture was observed. Certain specific features in the smooth newt's anti-predator behaviour were noted, setting it apart from that of the other two species (Table 1). All observed newts arched their bodies laterally. The hindlegs were spread, while the forelegs and front of the body were lifted from the ground. In low intensity postures, a weak bending of the body was followed by a coiling of the tail, while in high intensity postures the body was arched strongly, forcing the snout tip to touch the distal part of the cloaca. During such extreme displays the newts kept their eyes closed.

Some smooth newts stretched their bodies and elevated their tails while keeping them straight, without any undulating movement; sometimes the tail was lashed (Fig. 1h). This behavioural pattern was not observed in the other two species.

The combination of drift-fences and pit-falls is not a very selective method of capture; often other animals are trapped with the migrating newts. During the present study many shrews and voles were encountered in the traps (Mühlschlegel, 1994), as well as carabid beetles of the genus *Carabus* (Strupat, 1995).

TABLE 1. Description of anti-predator coloration and postures adopted in the field by three *Triturus* species; descriptions of postures follow Brodie (1977).

Species & sex/stage	no. of migrants	no. of obs.	aposematic colour		immobility	tail			body				venter exposed		
			venter	dorsum		lashed	wagged	undulated	arched	coiled	flipped	stretched	+tail up	+chin up	
<i>T. alpestris</i>															
males	3174	32	+	-	+	-	+	+	+	+	-	-	+	+	
females	3747	40	+	-	+	-	+	+	+	+	-	-	+	+	
juveniles	-	2	+	-	+	-	+	-	+	+	-	-	+	+	
<i>T. cristatus</i>															
males	51	6	+	-	+	-	+	+	+	+	-	-	+	+	
females	73	5	+	-	+	-	+	+	+	+	-	-	+	+	
juveniles	-	1	+	-	+	-	+	-	+	+	-	-	+	+	
<i>T. vulgaris</i>															
males	3182	26	+	-	+	+	-	-	+	+	-	+	+	+	
females	3879	37	+	-	+	+	-	-	+	+	-	+	+	+	
juveniles	-	5	+	-	+	-	-	-	+	+	-	-	+	+	

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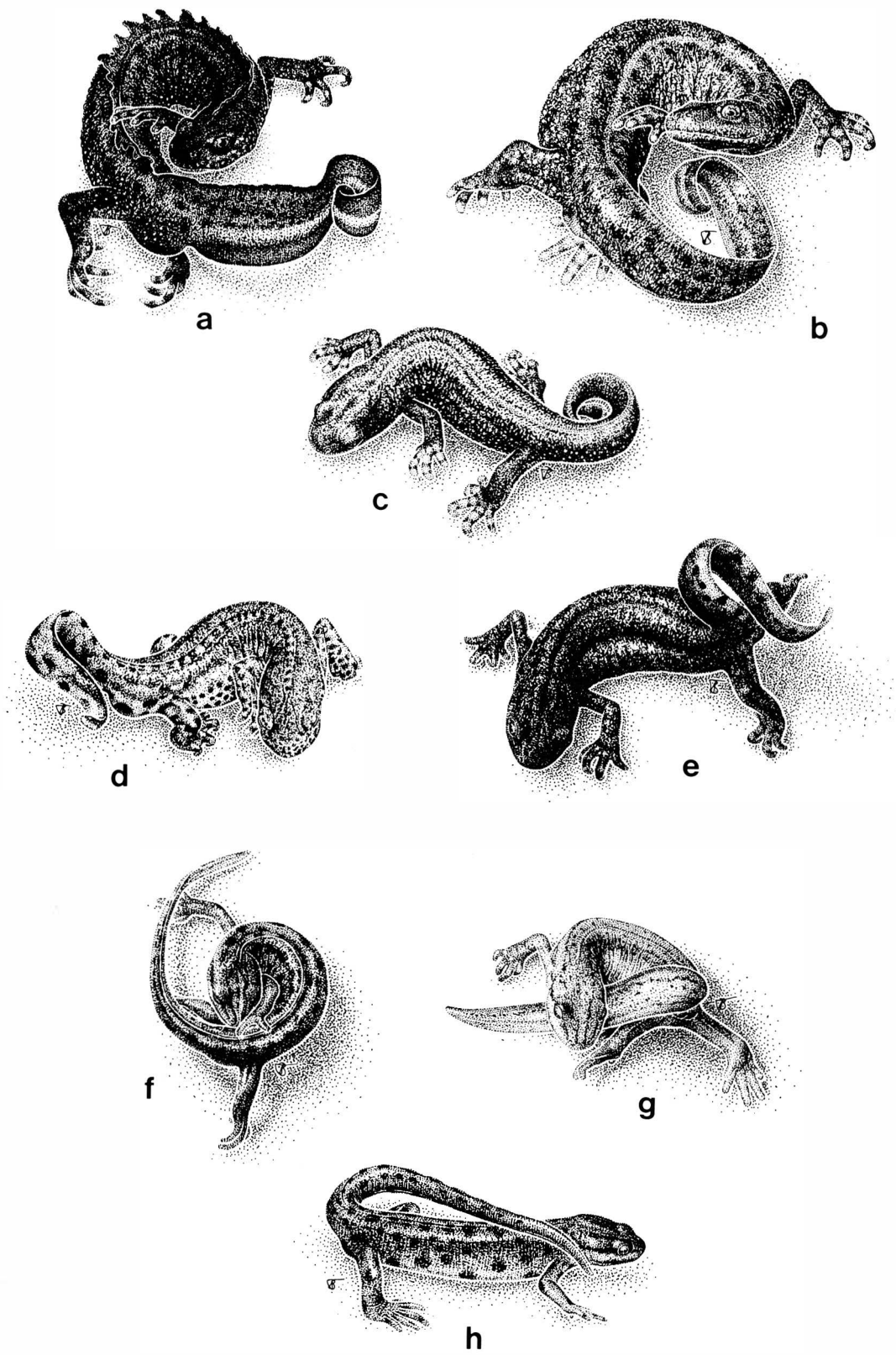


FIG. 1. Anti-predator behaviour of newts (genus *Triturus*): (a) *T. cristatus* male; (b) *T. cristatus* female; (c) *T. cristatus* juvenile; (d) *T. alpestris* male; (e) *T. alpestris* female; (f) *T. vulgaris* male; (g) *T. vulgaris* female; (h) *T. vulgaris* male, tail-lashing posture.

Shrews are known to prey upon smooth newts (Bell & Lawton, 1975; Pernetta, 1976). In addition, Thiesmeier (1990) identified carabids such as *Carabus nemoralis* and *Carabus hortensis* as predators of juvenile fire salamanders in pitfall traps. Therefore, the shrews and beetles trapped with the newts should be considered an important factor in inducing the newts' anti-predator postures.

Field records of anti-predator behaviour in the European *Triturus* species are rare and restricted to small numbers of animals, with few quantitative data available. Grillitsch (1983) described a female crested newt which had turned on its back, exposing its belly pattern. Denton (1990) induced a very strong defensive reaction in a female crested newt by grasping it firmly. Similar postures were observed in *Triturus carnifex* as it was removed from traps (Andreone, 1985). Alpine newts displaying anti-predator postures were observed by Zavadil (1992) and Kupfer (1995). For the smooth newt, no field data were available prior to the present note.

As mentioned above, some smooth newts showed an unusual anti-predator posture, in which the tail was displayed. A similar posture was described by Herrmann (1993) in *Triturus montandoni*. The tail of the smooth newt has a glandular dorsum (e.g. Nobis, 1949; Eber, 1954) and may be unpalatable, as we have observed a water shrew (*Neomys fodiens*) eating a male smooth newt, but leaving its tail. Displaying the tail might therefore serve to draw the predator's attention to this particular part of the body.

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REFERENCES

- Andreone, F. (1985). Observations on the defensive behavior of the crested newt (*Triturus cristatus carnifex*). *Brit. Herpetol. Soc. Bull.* **13**, 37-38.
- Bell, G. & Lawton, J. H. (1975). The ecology of the eggs and larvae of the smooth newt (*Triturus vulgaris* (Linn.)). *J. Animal. Ecol.* **44**, 393-423.
- Brodie, E. D. (1977). Salamander antipredator postures. *Copeia* **1977**, 523-535.
- Brodie, E. D., Nowak, R. T. & Harvey, W. R. (1979). The effectiveness of antipredator secretions and behavior of selected salamanders against shrews. *Copeia* **1979**, 270-274.
- Brodie, E. D., Nussbaum, R. A. & Digiovanni, M. (1984). Antipredator adaptations of Asian salamanders. *Herpetologica* **40**, 56-68.
- Denton, J. (1990). Defensive reflexes in newts of the genus *Triturus*. *Brit. Herpetol. Soc. Bull.* **32**, 30.
- Eber, G. (1954). Über die Morphologie von Hautdrüsenansammlungen bei Salamandriden. *Zool. Anz.* **152**, 153-162.
- Griffiths, R. A. (1996). *Newts and Salamanders of Europe*. London: Poyser.
- Grillitsch, H. (1983). Zum Feind-Abwehrverhalten des Kammolches, *Triturus cristatus cristatus* (Laurenti 1768). *Salamandra* **20**, 61-63.
- Herrmann, H.-J. (1993). Abwehrverhalten bei *Triturus montandoni* (Boulenger 1880). *Veröff. Naturhist. Mus. Schleusingen* **7/8**, 155.
- Hinsche, G. (1926). Vergleichende Untersuchungen zum sogenannten Unkenreflex. *Biol. Zentralblatt* **46**, 296-305.
- Kneitz, S. (1998). *Untersuchungen zur Populationsdynamik und zum Ausbreitungsverhalten von Amphibien in der Agrarlandschaft*. Bochum: Laurenti.
- Kupfer, A. (1995). Bemerkungen zum Abwehrverhalten des Bergmolches *Triturus alpestris alpestris* im Freiland. *Z. Feldherpetol.* **2**, 85-90.
- Kupfer, A. & Kneitz, S. (2000). Population ecology of the great crested newt (*Triturus cristatus*) in an agricultural landscape: dynamics, pond fidelity and dispersal. *Herpetol. J.* **10**, 165-171.
- Mühlschlegel, L. (1994). *Dynamik von Kleinsäugerzönosen im Drachenfelder Ländchen unter besonderer Berücksichtigung der Auswirkungen von Amphibien-Fanganlagen*. M.Sc. thesis, University of Bonn: Bonn.
- Nobis, G. (1949). Vergleichende und experimentelle Untersuchungen an heimischen Schwanzlurchen. *Zool. Jb. Abt. f. Anat.* **70**, 333-396.
- Pernetta, J. C. (1976). A note on the predation of the smooth newt *Triturus vulgaris* by the water-shrew *Neomys fodiens bicolor*. *J. Zool.* **179**, 215-216.
- Strupat, T. (1995). *Populationsökologische Untersuchungen zur Habitatwahl und Ausbreitung der Laufkäferarten Abax parallelipipedus (Piller et Mitterpacher) 1783, Carabus monilis Fabricius 1792, Carabus nemoralis Müller 1764 und Carabus problematicus Herbst 1786 (Coleoptera, Carabidae) in einer Agrarlandschaft*. M. Sc. thesis, University of Bonn: Bonn.
- Thiesmeier, B. (1990). Laufkäfer (Carabidae) erbeuten frisch metamorphosierte Feuersalamander (*Salamandra salamandra terrestris*) in Landfallen. *Salamandra* **26**, 218-220.
- Zavadil, V. (1992). Pozorváni obranné reakce colka horského, *Triturus alpestris* (Laurenti 1768). *Fauna Bohem. Septentr.* **17**, 85-87.

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AMPLEXUS-LIKE BEHAVIOUR OF HIBERNATING *RANA CHENSINENSIS* IN NORTHERN CHINA

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Key words: hibernation, Anura, breeding behaviour

Rana chensinensis David, 1875, was once regarded as a subspecies of *R. temporaria*, and more recently proposed as an independent species endemic to China (for taxonomic reviews see Pope & Boring, 1940; Liu & Hu, 1961; Xie *et al.*, 2000). Adapted to a cold climate and less-polluted water, the frog occurs widely in mountain rivers over northern China. So far, little has been published on the behavioural ecology of the species. During the winter period of 1992, I observed hibernation behaviour of *R. chensinensis* at two localities, Jie-xiu (37°04'N, 112°03'E; annual mean temperature 9.5°C, January mean -5.8°C) and Yu-she (37°32'N, 115°58'E; annual mean temperature 8.1°C, January mean -7.4°C), in Shanxi province, northern China. In Jie-xiu the frogs inhabited a small stream less than 2 m wide, surrounded by loess ravine with shrubs and farmland, while in Yu-she the frogs used a relatively complex river system encircled by woodland. I searched hibernation sites in each of the two study areas during the daytime. Once located, all individuals at a site were caught by hand as rapidly as possible. I determined the sex and age of the frogs by checking secondary sexual characters and measuring body length (SVL): sexually mature males had nuptial pads on the forefeet, while sexually mature females had a SVL > 35 mm in Jie-xiu and > 30 mm in Yu-she (Lu 1991, 1994). Then the frogs were released. In Jie-xiu, water temperatures at the bottom of the hibernating sites were measured on days when frogs were captured.

Like its close relatives, *R. temporaria* in Europe (Koskela & Pasanen, 1974) and *R. dybowskii* in north-eastern China (Huang, 1959; Ma, 1982; Li & Gao, 1984), *R. chensinensis* in the two study areas overwintered in groups in aquatic habitats. While hibernating, the frogs in Jie-xiu exhibited clear aggregation, with group size ranging from seven to 250 frogs (Table 1) in hollows under the stream bank and in warm springs. In contrast, in Yu-she the animals were relatively well-separated, under stones or other objects in the river, and the number of frogs found at any of the hibernation sites was less than seven individuals.

The most remarkable finding of the survey was that some individuals in hibernating groups showed amplexus-like behaviour. In this behaviour, a sexually

mature male clasped the chest of a sexually mature female with his forelimbs – just as in the breeding season, except that the male's hind legs were relaxed and the female was in a state of torpor with legs relaxed. No male-male, female-female or female-male clasping bonds were detected, except for two cases in which 4-5 individuals in a stream were found clasping each other in mid-November. I examined over 200 individuals along the stream in Jie-xiu during the pre-hibernation period (late-October) when they moved towards hibernation habitats, and I found no individuals clasped together. This indicates strongly that the amplexus-like behaviour was initiated early in the hibernation period (mid-November). The clasping was very tight and persistent. Several clasping frogs brought to the laboratory remained together for at least 48 hours. In Jie-xiu, I regularly visited two springs where the hibernating frogs could be seen in the clear water. Clasping frogs were observed there from early until late in the hibernation period (i.e. early November until mid-January). I am not certain whether clasping bonds changed during the hibernation period, owing to the lack of marked individuals, but in both the cold stream covered with ice (near 1°C) and the warm springs (about 10°C), the frogs remained inactive and in a state of torpor throughout the wintering period. Therefore, although a few frogs were occasionally found swimming in spring habitats, it seems likely that those clasping-bonds formed early in the hibernation period could be relatively stable.

Over a four-year period the earliest egg-laying took place in mid-February for the frog population in Jie-xiu, and in mid-March in Yu-she (X. Lu personal observation). During the hibernating period, despite the relatively high spring water temperature that allows spawning, the frogs exhibited no other behaviour associated with reproduction. Perhaps the low air temperature is ultimately a limiting factor. Thus, the amplexus-like behaviour was not directly associated with reproduction. One possible explanation under consideration is avoidance of male-male competition for mating in the subsequent spawning period. It has been shown that for explosive breeders, with a short breeding season, the male-male competition for mates is remarkable (Wells, 1977). In *R. temporaria*, male-male competition is not intense enough to lead to non-random mating in natural populations with operational sex ratio (OSR) more than 0.5, but it may be so in experimental populations with a heavily male-biased OSR and high male density (Elmberg, 1991). In my data (Table 1), no significant correlation was found either between occurrence of hibernation clasping and hibernating group size ($r_s = 0.502$, $df = 11$, $P = 0.115$) or between occurrence and OSR ($r_s = -0.191$, $df = 11$, $P = 0.573$). Furthermore, the higher OSRs of the breeding population in the study areas, where spawning lasts for about six weeks (Jie-xiu: 77/87=0.89; Yu-she: 79/43=1.84, X. Lu personal data) seemed not to suggest the occurrence of intense male-male competition. Comparing the body lengths of eight males (37.7±1.6 mm) and eight females (39.0±1.7 mm) in-

TABLE 1. Group size, sex ratio and occurrence of amplexus-like behaviour in different hibernating refuges of *Rana chensinensis* in northern China. ¹The number of sexually-immature frogs is given in parentheses. ²Value of sexually-mature specimens. ³Number of clasping-bonds/number of females x 100. *Pooled data comprising specimens caught at different hibernating refuges, with 1-7 sexually mature frogs at any one.

Date	Winter refuge	Water temperature (°C)	Group size ¹	Female	Male	Sex ratio ² (female/male)	Clasping-bond	
							no.	%females ³
(1) <i>Jie-xiu</i>								
19 Nov	Stream	1.5	20 (0)	5	15	0.33	2	40.3
20 Nov	Stream	1.0	25 (0)	8	17	0.50	0	0.0
20 Nov	Stream	1.0	50 (0)	18	32	0.57	2	11.0
20 Nov	Stream	1.2	140 (8)	59	81	0.73	8	13.5
21 Nov	Stream	1.7	11 (0)	3	8	0.38	0	0.0
21 Nov	Stream	1.5	14 (0)	4	10	0.40	1	25.0
25 Nov	Spring	11.2	249 (6)	71	178	0.40	25	35.1
15 Dec	Spring	10.5	43 (1)	25	18	1.33	1	4.1
16 Dec	Spring	10.5	131 (3)	60	71	0.85	45	74.8
17 Jan	Stream	1.2	21 (0)	8	13	0.62	0	0.0
17 Jan	Stream	1.0	13 (2)	7	6	1.20	0	0.0
19-25 Nov	Stream	—	22 (4)*	6	16	0.38	0	0.0
15-16 Dec	Stream	—	31 (4)*	10	21	0.48	0	0.0
(2) <i>Yu-she</i>								
9-12 Dec	Stream	—	67 (19)*	37	30	1.23	8	21.6

volved in clasping in Yu-she with those of 29 sexually-mature males (36.5 ± 0.8 mm) and 22 females (38.2 ± 1.1 mm) hibernating singly, I found no significant difference either between males ($t=0.70$, $df=35$, $P=0.49$) or between females ($t=0.40$, $df=28$, $P=0.69$). This suggests that the amplexus-like behaviour occurred at random with regard to body size. Therefore, to explain the adaptive significance of the amplexus-like behaviour during hibernation, further study should estimate the reproductive success of the individuals involved in the clasping-bonds.

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REFERENCES

- Elmberg, J. (1991). Factors affecting male yearly mating success in the common frog, *Rana temporaria*. *Behav. Ecol. Sociobiol.* **28**, 125-131.
- Huang, Z. J. (1959). Migration and wintering of *Rana temporaria chensinensis*. *Chinese J. Zool.* **8**, 363-364.
- Koskela, P. & Pasanen, S. (1974). The wintering of the common frog, *Rana temporaria* L., in northern Finland. *Aquilo Ser. Zool.* **15**, 1-17.
- Li, J. L. & Gao, Z. Y. (1984). A preliminary observation on ecology of *Rana temporaria chensinensis*. *Nat. Rec. Res.* **4**, 49-51.
- Liu, C. Z. and Hu, S. Q. (1961). *Chinese Amphibia*. Beijing: Science Press.
- Lu, X. (1991). The seasonal change of body length and weight of adult *Rana chensinensis* in Taiyue mountain. *Sichuan J. Zool.* **10**, 32-33.
- Lu, X. (1994). Feature of fecundity and its geographic variation in *Rana chensinensis*. *Acta Ecol. Sin.* **14**, 209-214.
- Ma, C. F. (1982). Studies on reproductive ecology of *Rana temporaria chensinensis*. *Acta Herp. Sin.* **1**, 48-62.
- Pope, C. H. & Boring A. M. (1940). A survey of Chinese Amphibia. *Pek. Nat. Hist. Bull.* **15**, 13-86.
- Wells, K. D. (1977). The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666-693.
- Xie, F., Ye, C. Y., Fei, L., Jiang, J. P., Zeng, X. M. & Matsu, M. (2000). Taxonomical studies on Brown frogs (*Rana*) from northeastern China (Amphibia: Ranidae). *Acta Zootax. Sin.* **24**, 224-231.

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**DIET AND MORPHOMETRICS OF
COLUBER (=HIEROPHIS)
VIRIDIFLAVUS ON THE ISLAND OF
MONTECRISTO (TYRRHENIAN SEA,
ITALY)**

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Key words: Coluber, snake, feeding, ecology

The European whip snake, *Coluber* (= *Hierophis*) *viridiflavus* is a common colubrid snake of the Mediterranean region, ranging from western and N.E. France to Switzerland, and to Italy and most of the western Mediterranean islands. It inhabits a great variety of habitats and ranges up to 2000 m asl (Naulleau, 1997). This species is characterized by a large body size, up to 200 cm in length (Bruno & Maugeri, 1990), and has a widespread distribution and habitat use (Bruno & Maugeri, 1990; Scali & Zuffi, 1994). The European whip snake consumes a wide variety of prey (Bruno & Maugeri, 1990; Luiselli & Angelici, 1996; Capula, Filippi, Luiselli & Trujillo Jesus, 1997), and also displays an ontogenetic shift in diet composition (Bruno & Maugeri, 1990; Rugiero & Luiselli, 1995), as typically observed in several other snake species (Luiselli & Agrimi, 1991; Luiselli, Capula & Shine, 1996). Of particular interest is the ecology (i.e. feeding ecology, thermal preferences), and activity of this species in areas or habitats where potential competitors are limited in number or absent (e.g. small islands), compared with areas where potential competitor species are common and abundant (e.g. natural parks of coastal areas, large islands). The feeding ecology of *C. viridiflavus* on the Mediterranean islands is still incompletely known, and further research and additional data could be useful to confirm most patterns of the phenotypic plasticity observed (Bruno, 1968, 1975; Delaugerre & Cheylan, 1992). The island of Montecristo was chosen for study because of the presence of only one other, competitor snake species, *Vipera aspis hugyi* (Societas Herpetologica Italica, 1996) and because of the very small size of the island. It is generally assumed that in restricted or closed habitats the amount of food resources can fluctuate markedly in time, as also can the relative predator density (Andrén & Nilson, 1983). In this short note, I present and discuss (1) new data on diet, and (2) additional natural history data for *Coluber* (= *Hierophis*) *viridiflavus*.

During a five-year research project on integrated ecology and systematic aspects of the Mediterranean snake fauna, it was possible to visit the Natural Reserve of the island of Montecristo, an area highly protected

for breeding and migratory birds (Meschini & Frugis, 1993), and for the presence of a large population of the wild goat (*Capra aegagrus hircus*), a naturalized species introduced by pre-historic man probably 6000 years B.C. (Masseti, 1993). This small island is about 10 km² in area, mainly granitic, with typical Mediterranean vegetation, comprising a small woodland of introduced *Pinus pinea*, and sparse autochthonous relict *Quercus ilex* trees. The average temperature in July is about 24 °C (Pavan, 1989). The research was carried out from 0630 hr to 2030 hr, during the week of 5-11 June 1999, along a 1450 m long and 300 m wide transect, selected as a representative selection of habitat patterns on the island. The transect ran from the sandy beach at sea level in the west, eastwards through a pine-wooded area, and along a temporary stream with permanent small ponds and ended at the medium-altitude, bush-covered central part of the island. The transect was walked three times a day, at 0700 hr, 1100 hr (in the opposite direction), and 1700 hr. The starting point of the first survey alternated between days, i.e. on one day it was at the coast, while on the the following day it was at the central part of the island. Each transect survey lasted about three hours. Meteorological data were taken from 0400 to 2400 hrs, on average at two hour intervals; shaded air temperature at 10 cm above ground and relative humidity were recorded; data were presented as average \pm 1 SD and range (minimum-maximum values, sample size). Snakes were captured between about 100 m and more than 1200 m away from the coast, and from 10 m to 240 m asl. Snout-vent length (SVL, precision 1 mm), total length (TL, precision 1 mm), number of ventral scales (VS), body mass (BM, precision 0.5 g) and tail condition (0 = intact tail, 1 = damaged tail) and sex for each captured snake were recorded. Each morphometric variable was log-transformed prior to the analyses and tested for normality, then processed with parametric or non-parametric statistics, according to a normality test. Statistical analyses were carried out with SPSS 6.1.2.

Average air temperature (mean of daily means) during the study was 25.2 \pm 1.8 °C (21.8-30.4, $n=46$), and average relative humidity was 59 \pm 11.4 % (34-82, $n=33$). I observed 17 adult and two subadult snakes, and captured 11 of them – seven adult and one subadult males and three adult females. In 17 out of 19 encounters, the snakes were very close to the water (i.e. less than 2 m distance, $n=7$) or directly in the water ($n=10$), either basking or searching for food. Two snakes were close to areas of bushes and rocks, more than 100 m from the closest humid area. Air temperature at the time of capture was 25.61 \pm 1.32 °C (24-27.6; $n=9$), ground temperature was 28.25 \pm 2.75 °C (25.6-33; $n=8$); these values appear to be higher than those recorded during the limited available field records for basking activity of *Coluber* (= *Hierophis*) *viridiflavus* in northern Italy (Scali & Zuffi, 1994) and central Italy (Capula *et al.*, 1997). They are, however, similar to those in the only available report for the species on this island, with a range at capture of 16.6-33.4 °C (Bruno, 1975: page 78). Fourteen whip snakes were encountered between 0800

TABLE 1. Biometric and dietary data for *Coluber* (= *Hierophis*) *viridiflavus* from the island of Montecristo. Sex: M = male, F = female; tail: 0 = intact, 1 = damaged. For further explanations see text.

snake no.	sex	total length (mm)	SVL (mm)	No. ventrals	Tail	Food remains
1	M	107	77	204	0	no data
2	M	89	79	210	1	no data
3	M	107	78.5	205	0	<i>Discoglossus</i> (femur, ribs); formicidae; tenebrionidae
4	M	91	66.5	214	0	no data
5	M	88	64.5	209	0	<i>Discoglossus</i> (vertebrae, ribs); formicidae; tenebrionidae
6	M	116	85	206	1	no data
7	M	107	78	204	0	<i>Discoglossus</i> (vertebrae); formicidae; Coleoptera indet.
8	M	/	55.4	207	0	Empty
9	F	60	55.4	222	1	<i>Discoglossus</i> (skin)
10	F	95	71.9	207	0	Empty (gravid female, three developing eggs)
11	F	104	77	219	0	no data

and 1259 hrs (no. captured=8), and five were encountered between 1730 and 1810 (no. captured=3). These observations suggest a bimodal cycle of daily activity which is probably common during the hot months (Capula *et al.*, 1997: page 72), although most activity patterns in snake species can be fully explained only from prolonged field studies or by using radio tracking procedures (Ciofi & Chelazzi, 1991; Naulleau, 1992).

Considering the relative scarcity of biometric data on the whip snake from Mediterranean islands (Bruno, 1968, 1975; Schätti & Vanni, 1986; Delaugerre & Cheylan, 1992; Zuffi, 2000), all raw data on specimens captured during the present study are given in Table 1. Three out of eight males and one out of three females had damaged tails; this is consistent with a relatively high incidence of attempted predation on this island population, or perhaps an effect of parasites that may destroy the tail (personal observation). Alternatively, it could have resulted from natural tail breakage, which occasionally occurs in snakes (Mendelson III, 1992). Owing to the reduced TL sample size, it was impossible to perform any meaningful statistical analysis on this variable. In addition to 11 adult snakes captured during this study, I have added measurements of 16 adults (nine males and seven females) reported by Bruno (1968: page 53). Male whip snakes measured 741 ± 98 mm SVL (534–873 mm, $n=17$), weighed 76.6 ± 0.7 g BM ($n=8$), and had 205.9 ± 3.6 VS (200–214, $n=19$); females measured 681 ± 79 mm SVL (554–790 mm, $n=10$) and had 217.4 ± 6.0 VS (207–230, $n=10$); the two females weighed had a BM of 65 g and 77 g, respectively. Adult SVL did not differ significantly between the two sexes ($t=1.6$, $df=25$, $P=0.122$), whereas males had fewer ventral scales ($t=6.48$, $df=27$, $P=0.0001$). Data presented by Schätti & Vanni (1986: Fig. 1, on page 223) for the Montecristo whip snakes overlap the data in this study. Furthermore, both data sets confirm that *C. viridiflavus* of Mediterranean islands are characterized by smaller body size and greater number of VS than those of mainland Italy. According to Schätti & Vanni (1986), island males average 780–895 mm SVL with 203–206 VS whereas mainland

males average 830–1089 mm SVL with 194–201 VS; island females average 784–820 mm SVL with 217–223 VS whereas mainland females average 700–909 mm SVL with 205–213 VS. The results confirm the previously observed sexual dimorphism of this taxon (Springolo & Scali, 1998), and that small-island whip snakes have, on average, a greater number of ventral scales and smaller body size than mainland populations (Schätti & Vanni, 1986; Delaugerre & Cheylan, 1992; Zuffi, 2000; Zuffi, Corti & Luiselli, 2000).

Smaller body size and a greater number of ventral scales in small island populations is a pattern often found in this taxon (Schätti & Vanni, 1986; Delaugerre & Cheylan, 1992; Zuffi *et al.*, 2000), and quite often in other colubrid species (Corti, Zuffi & Luiselli, 2000; Zuffi *et al.*, 2000). A smaller body size could be favourable in terms of a reduced energy requirement, but it may also be involved in a different reproductive strategy (Zuffi, 2000; Zuffi *et al.*, 2000). Alternatively, a smaller body size may be interpreted as a result of low energy availability, not necessarily indicating any adaptive pattern.

I examined the intestinal contents of three of the 11 adult snakes that were kept for two days in enclosures, and of another three that were retained as voucher material according to the EU INTERREG II project Corsica-Tuscany. *Discoglossus sardus*, the only amphibian species present on Montecristo (Corti *et al.*, 1991), was the only vertebrate prey found (Table 1); the insects found in the food remains had almost certainly been eaten by the *Discoglossus* themselves. This observation was very different to those of Bruno (1968, 1975), who found that in May and July 1967 whip snakes on Montecristo had eaten small mammals (*Apodemus*), birds (*Motacilla*, *Phylloscopus*, *Phoenicurus*, *Muscicapa*, *Hippolais*), lizards (*Podarcis*) and their eggs, and locusts (*Locusta*). There was only one occurrence of *Discoglossus* in the diet of *C. viridiflavus* (Bruno 1968, 1975). Dietary data for *Coluber* (= *Hierophis*) from Montecristo are still scarce. The high incidence of tadpoles and adults of *Discoglossus* in the diet during my study may have reflected opportunistic

exploitation by the snakes of a seasonally abundant resource, or it may have resulted from the snakes seeking out water during a period of high temperature. Whether the snakes selected wet areas because of the hot weather, then fed on the most available prey, or selected wet areas because of the seasonal abundance of amphibian prey raises a more general question about the forces underlying prey selection by this species – a topic worthy of further research.

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REFERENCES

- Andrén, C. & Nilson, G. (1983). Reproductive tactics in an island population of adders, *Vipera berus*, (L.), with a fluctuating food resource. *Amphibia-Reptilia* **4**, 63-79.
- Bruno, S. (1968). Gli Anfibi e i Rettili dell' Isola di Montecristo (Studi sulla fauna erpetologica italiana, IX). *Atti Soc. Tosc. Sc. Nat. Mem. B*: **75**, 31-71.
- Bruno, S. (1975). Note riassuntive sull'erpetofauna dell'Isola di Montecristo (Arcipelago Toscano, Mare Tirreno) (Studi sulla fauna erpetologica italiana, XX) (Studi sulla Riserva Naturale dell'Isola di Montecristo, XVIII). *Lavori Soc. Ital. Biogeog.* **5** (1974), 743-838.
- Bruno, S. & Maugeri, S. (1990). *Serpenti d'Italia e d'Europa*. Milano: Mondadori.
- Capula, M., Filippi, E., Luiselli, L. & Trujillo Jesus, V. (1997). The ecology of the Western Whip snake, *Coluber viridiflavus* (Lacépède, 1789), in Mediterranean Central Italy (Squamata: Serpentes: Colubridae). *Herpetozoa* **10**, 65-79.
- Ciofi, C., & Chelazzi, G. (1991). Radiotracking of *Coluber viridiflavus* using external transmitters. *J. Herpetol.* **25**, 37-40.
- Corti, C., Zuffi, M. A. L., & Luiselli, L. (2000). Sulla variazione geografica e biometria comparata della natrice dal collare, *Natrix natrix*, dell'area Mediterranea. 3° Congr. S.H.I., Pavia, 14-16 settembre 2000, *Riassunti*, 1 pag.
- Corti, C., Nistri, A., Poggesi, M. & Vanni, S. (1991). Biogeographical analysis of the Tuscan herpetofauna (Central Italy). *Rev. Esp. Herp.* **5**, 51-75.
- Delaugerre, M. & Cheylan M. (1992). *Atlas de repartition des Amphibiens et Reptiles de Corse*. Pamplona: Oikema.
- Luiselli, L. & Agrimi, U. (1991). Composition and variation of diet of *Vipera aspis francisciredi* in relation to age and reproductive stage. *Amphibia-Reptilia* **12**, 137-144.
- Luiselli, L. & Angelici, F. M. (1996). The prey spectrum of terrestrial snakes in the Tolfa Mountains (Latium, Central Italy). A synthesis from earlier analysis. *Herpetozoa* **9**, 111-119.
- Luiselli, L., Capula, M. & Shine, R. (1996). Reproductive output, costs of reproduction, and ecology of the smooth snake, *Coronella austriaca*, in the eastern Italian Alps. *Oecologia* **106**, 100-110.
- Meschini, E. & Frugis, S. (1993). Atlante degli uccelli nidificanti in Italia. *Suppl. Ric. Biol. Selvaggina* **20**, 1-344.
- Masetti, M. (1993). Post-Pleistocene variations of the non-flying terrestrial mammals on some Italian islands. *Suppl. Ric. Biol. Selvaggina* **21**, 209-217.
- Mendelson, J. R. (1992). Frequency of tail breakage in *Coniophanes fissidens* (Serpentes: Colubridae). *Herpetologica* **48**, 448-455.
- Naulleau, G. (1992). Activité et température corporelle automnales et hivernales chez le Couleuvre d'Esculape *Elaphe longissima* (Squamata, Colubridae) dans le Centre Ouest de la France. *Bull. Soc. Herp. Fr.* **64**, 21-35.
- Naulleau, G. (1997). *Coluber viridiflavus* (Lacépède, 1789). In: Gasc J. P. et al. (Eds.). Atlas of Amphibians and Reptiles in Europe. Societas Europaea Herpetologica & Muséum National d' Histoire Naturelle (IEGP/SPN), Paris: 342-343.
- Pavan, M. (1989). *Isola di Montecristo. Riserva Naturale*. Collana verde 77/1989. Rome: Ministero Agricoltura e Foreste, Corpo Forestale dello Stato.
- Rugiero, L. & Luiselli, L. (1995). Food habits of the snake *Coluber viridiflavus* in relation to prey availability. *Amphibia-Reptilia* **16**, 407-411.
- Scali, S. & Zuffi, M. (1994). Report on a reptile community ecology in a suburban habitat of northern Italy (Reptilia, Sauria, Serpentes). *Boll. Zool.* **61**, 73-76.
- Schätti, B. & Vanni, S. (1986). Intraspecific variation in *Coluber viridiflavus* Lacépède, 1789, and validity of its subspecies (Reptilia, Serpentes, Colubridae). *Revue suisse Zool.* **93**, 219-232.
- Societas Herpetologica Italica (1996). Atlante provvisorio degli Anfibi e dei Rettili italiani. *Annali Mus. Civ. St. Nat. "G.Doria"* **91**, 95-178.
- Springolo, M. & Scali, S. (1998). Sexual dimorphism and ontogenetic changes in *Coluber viridiflavus*: a head morphometric analysis. In: Miaud, C. & Guyétant R. (Eds.). Societas Europaea Herpetologica, Le Bourget du Lac, France. 1998: 413-417.
- Zuffi, M. A. L. (2000). Morphological and functional variability of the whip snake (*Hierophis viridiflavus*) from Corsica: a comparative approach to other Mediterranean islands and mainland. *Ambiente e Identità nel Mediterraneo, Bastia, Ajaccio, Corte 13-16 Giugno 2000*, Proceedings, 5 pp.
- Zuffi, M. A. L., Corti, C. & Luiselli, L. M. (2000). Significato adattativo della variabilità morfologica nel biacco (*Hierophis viridiflavus*) del Mediterraneo. 61° Congr. UZI, S. Benedetto del Tronto (AP), 24-28 settembre 2000, *Riassunti*, 1 p.

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BOOK REVIEWS

Tadpoles. The Biology of Anuran Larvae. Roy W. McDiarmid & Ronald Altig (eds.) (1999). University of Chicago Press, Chicago and London. £44.50 (cloth), £25.50 (paper).

Traditionally, the biological cake is cut in two ways, with taxa in the slices (e.g. birds, amphibians, reptiles) and topics in the layers (e.g. physiology, ecology, evolution). Consequently, the vast majority of books address a subject from one or both of these angles. Producing a book about one life-stage of a taxonomic group is a less popular approach, presumably because it is almost impossible to write a cohesive account on, say, the topic of eggs, without embracing the reproductive cycles and general biology of the adult stages that produce them. Likewise, how can it be possible to produce a book on the topic of tadpoles without encompassing the other phases of anuran life history that are intrinsically linked to them? However, this book is about much more than just a curious stage in the anuran life cycle. It is, in fact, about how whole issues in amphibian life history and evolution can be addressed, using tadpoles as model organisms.

The twelve chapters that comprise the book are the work of some fourteen contributors (all but three of whom are based in the USA), with the editors providing the first three and the final chapters. The brief introductory chapter, in which McDiarmid and Altig focus mainly on historical aspects of tadpole research, makes for refreshing – and in places entertaining – reading. Some intriguing statistics underline how tadpole research has developed over the years. About 72% of all papers on tadpole biology have been published since 1980, and 25% since 1990, so here we have a young and apparently blossoming science. In the second chapter the same authors go on to discuss research materials and techniques, and provide a useful tidying-up of the often confused terminology and criteria for staging tadpoles. Of all the chapters in the book, this will have the greatest appeal to the non-scientist: the sound advice provided here on such aspects as catching, photographing and rearing will be of use to both the field naturalist and captive breeder. Chapters 3–7 cover morphological and anatomical aspects, in the form of solid comprehensive essays on the musculoskeletal, endocrine, and nervous systems, as well as on the development and evolution of endotrophy. Environmental physiology receives a very thorough review by Gordon Ultsch in chapter 8. It is interesting to see that the work carried out by R. Maxwell Savage between the 1930s and 1960s on *Rana temporaria* in England is still influential, although research since that time has been dominated by that on North American species. This raises some interesting comparisons with British species. For example, one general pattern is that bufonid tadpoles are lungless prior to metamorphosis, while ranid tadpoles have functional lungs from an early stage

of development that allows them to gulp air from the surface. The environmental correlates of this are that North American bufonids tend to breed in shallow, temporary ponds that have high levels of dissolved oxygen, while ranids are found more often in more permanent water bodies with lower oxygen levels. No mention is made of the fact that the opposite pattern is usually observed in British *Bufo bufo* and *Rana temporaria*, so perhaps the habitat-physiological correlates of this species pair would be worth pursuing in this context. Indeed, the influence of research conducted in North America on amphibian biology is evident throughout the book. Table 8.2, for example – which summarizes work on thermoregulation in tadpoles – cites literature almost exclusively from that continent. As in many other areas of tadpole biology, research on thermoregulation has focused predominantly on temperate species, whereas the greatest diversity of anurans occurs in the tropics. Despite these inherent and unavoidable biases, a general point that seems to emerge is that, compared to other ectothermic vertebrates, the temperature tolerance ranges of tadpoles are remarkably wide.

Behaviour and ecology are dealt with in chapters 9 and 10 respectively. Studies of the sensory and communication systems of tadpoles have provided some fascinating insights into problems of more general evolutionary significance. Kin recognition and kin association, for example, is one field where work on tadpoles has made some fundamental contributions to ethology, and anyone starting work in this area – whether or not they are interested in tadpoles – would be advised to consult the comprehensive and up to date account provided here by Hoff, Blaustein, McDiarmid and Altig. There are also some interesting snippets of information here that surprised me. I was not aware, for example, that tadpoles are generally short sighted. Ecological studies of tadpoles have also shed light on problems of more general relevance, and the comprehensive review provided in chapter 10 by Ross Alford underlines this. Such is the volume of literature on certain topics that this chapter contains lengthy sections that remain unbroken by subheadings, which makes for a rather exhausting read. Much of the focus of this chapter boils down to the interaction between tadpoles and the resources upon which they depend, and I was intrigued to discover here that in some ponds in Carolina, tadpole faeces forms a layer several centimetres thick. The Wilbur-Collins model of amphibian metamorphosis has been the cornerstone of much tadpole biology since the 1970s, and is extensively reviewed here, along with community structure, density effects and intra- and interspecific competition.

Several topics that have already been touched on in previous chapters are revisited in chapter 11, which covers evolution and maintenance. Aspects of growth and development feature prominently here, with subsequent tests and modifications of the Wilbur-Collins model receiving a thorough airing. The author, Reid Harris, presents a new graphical model showing how energy

may be apportioned to growth and development, and discusses the genetic basis of these parameters. He challenges the traditional view that recently-filled temporary ponds provide a rich flush of primary productivity. Instead, he argues that food resources may be at their highest as the pond dries and nutrients become concentrated. All of these issues are discussed within the context of the evolution of complex life cycles.

The final chapter, entitled 'Diversity', is a comprehensive family-by-family summary of the morphological features of tadpoles. The descriptions are accompanied by comprehensive illustrations, with useful supplementary information on the number of genera and species in each family, together with its geographical range. The book concludes with a glossary of tadpole terminology, and nearly sixty pages of cited literature. Both author and subject indices are provided to assist the reader in navigating this extensive volume, and there are useful summaries provided at the end of each chapter.

For researchers new to the field of tadpole biology this comprehensive text will be the standard starting point for many years to come. However, the breadth and depth of the coverage also mean it is an essential companion for the seasoned herpetologist. Assembling such a vast wealth of detailed information under one cover must have been a daunting task for the editors. In particular, such is the interweaved nature of tadpole biology that deciding how the field should be meaningfully divided up between stand-alone chapters must have required considerable soul-searching. The fact that some topics are addressed from different angles across several chapters makes this a book that needs to be read and reflected upon rather than simply dipped into as a reference guide. I did feel that had the pool of contributors been a little more international, the emphasis of certain chapters might have been slightly different. Nevertheless, the editing of this book has been meticulous and the production standards are immaculate – the numerous line drawings, graphics and photographs have all been reproduced with a consistent clarity that is often missing in more expensively produced books. Indeed, the price of the paperback edition which was published last year is very competitive for a specialist academic text book.

It would be churlish to try and find fault with a work of this magnitude. My only regret is that this book was not available to me some twenty years ago when I started dabbling in tadpole ecology. It would have saved me many months of lonely literature-trawling in the bowels of numerous libraries, thus freeing me to indulge in what the editors of this book call 'the elixir we know as tadpole field biology'. McDiarmid and Altig both confess to succumbing to this potent force early in their careers. There will be very many herpetologists who will be eternally thankful that they did.

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Die Waldeidechse: unscheinbar – anpassungsfähig – erfolgreich. Dieter Glandt. (2001). Bochum: Laurenti-Verlag, 111 pp. Zeitschrift für Feldherpetologie: Beiheft 2. DM 39.80.

For those not familiar with the German language, the title of this short book translates as: *The common lizard: inconspicuous – adaptive – successful*. The three adjectives of the sub-title neatly describe the most important features of this "common" species: it is not sufficiently attractive to warrant special attention, it shows a marvelous adaptability to a great variety of conditions and, in view of its enormous distribution area, it is one of the most successful reptiles of this era.

In some 13 chapters the author – who has studied reptiles and amphibians for more than 30 years – creates a comprehensive picture of this peculiar lizard. In the first two short chapters the motives for writing a book on such a common species are given, followed by a summary table of its general features. Morphological characteristics, such as weights and measures, are presented, together with information on the remarkably different chromosome numbers found in different regions. Chapter 4 depicts the huge distribution area of the species, while chapter 5 describes the highly varied habitats that it occupies. This is followed by a treatment of its physiological capacities in chapter 6, focusing on thermoregulation, water balance and freeze tolerance. Chapter 7 concentrates on feeding and energy balance, while chapter 8 gives an overview of annual and daily activities, migrations and orientation mechanisms. In chapter 9 the peculiar reproductive biology of this lizard is dealt with, referring to the combination of viviparous as well as oviparous reproduction modes. Chapter 10 presents a treatment of the taxonomic position of the species and descriptions of subspecies. Then follows a longer chapter on population ecology, with sections on population dynamics, sex ratio, fertility, longevity and the influence of predators and parasites. Chapter 12 discusses threats and conservation; chapter 13, capture methods and individual marking techniques. All this is concluded by a list of 268 references and a short glossary of terms and names used.

This publication presents a nice synthesis of investigations on the viviparous lizard. It is written in German, but the author also drew on results published in other languages from throughout Europe. It is written in a stimulating way, inviting new research in certain areas where problems are not yet solved. As such it is essential reading for everyone involved in the study of this animal or closely related species. Equally, the completeness of the coverage makes it highly recommended to others who want to form a picture of this particular "commoner".

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THE HERPETOLOGICAL JOURNAL

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(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.
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5. The usual rules of zoological nomenclature apply.
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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. (1969a). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

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