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FRONT COVER: Physignathus cocincinus (R. Meek)

THERMOREGULATION AND ACTIVITY PATTERNS IN CAPTIVE WATER DRAGONS, *PHYSIGNATHUS COCINCINUS*, IN A NATURALISTIC ENVIRONMENT

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Observations were made on the thermoregulatory behaviour and activity patterns of *Physignathus cocincinus* in a simulated tropical environment. *P. cocincinus* is a sit-and-wait predator with movement restricted to a maximum of 9 or 10 bursts of activity per day. Both terrestrial and aquatic activity were temperature dependent, and were greatest in hot, sunny weather. When the weather was overcast, the lizards spent most of their time in open areas and at higher levels in the canopy; increased sunshine induced movement to lower levels, with more time spent in shade. Regression analysis of the relationship between lizard and model temperatures was used to determine thermoregulation. *P. cocincinus* is basically a thermoconformer, but needs to thermoregulate on sunny days to avoid excessive heat loads. Control of body temperature during hot and intermittent sunshine was principally achieved by seeking shade and partial basking. On cloudy days the animals were thermoconformers, as environmental temperatures were sufficiently high to enable locomotory activity and feeding to take place, but not so high that excessive heat loads became a problem.

Key words: Physignathus cocincinus, thermoregulation, activity patterns

INTRODUCTION

Much of the early work on reptile thermoregulation concerned lizards from temperate or desert habitats (see Avery, 1979, 1982; Bradshaw, 1986 for reviews). These studies have given rise to a model of lizards as active sunbaskers, usually with precise body temperatures controlled by an elaborate series of shuttling or posturing behaviours. However, later research on lizards from forests and other densely vegetated environments described species with much broader activity temperatures, and with only occasional basking behaviour or none at all (e.g. Huey & Slatkin, 1976; Huey, 1982; Avery, 1982). Most of these studies have dealt with small lizards (<50 g); relatively few works have examined the thermal biology of the larger reptiles, including those from tropical forests. This paper is concerned with the thermal biology and activity patterns of a medium-sized, tropical forest lizard, the green water dragon, Physignathus cocincinus. Very little is currently known about the thermal ecology of P. cocincinus, despite its popularity as a zoological exhibit and presence in the pet trade. The only thermal studies are from Australian Physignathus, with field and laboratory body temperatures being recorded from P. longirostris (Licht et al., 1966) and P. lesueurii (Grigg et al., 1979).

The green water dragon, *P. cocincinus*, is a sit-andwait predator feeding on small vertebrates and insects. The species has an extensive distribution over southeast Asia and Indochina, where it lives in and around dense vegetation alongside rivers and other aquatic areas. Such environments are generally thermally stable, deviating only slightly from an annual mean of 25°C, with a wet season that lasts for several months each year. In this type of habitat, accurately measuring body temperature and continuously observing behaviour, including the habit of *P. cocincinus* of diving into water at the approach of danger, are problematical. However, in a spacious, simulated natural environment, captive animals may provide ideal subjects for detailed, continuous observation.

There has been some debate about how thermoregulatory data in reptiles should be interpreted. Early studies used the criterion of body temperatures being elevated above air temperatures as evidence of thermoregulation (Cowles & Bogert, 1944), but subsequent demonstration of the flaws of this method (Heath, 1964) prompted the use of more elaborate statistical methods. Regression analysis of body temperatures and environmental temperatures (Huey & Slatkin, 1976) provided a standard theoretical and statistical approach, although it has been suggested more recently that a reptile may not necessarily be thermoconforming when its body temperature is in agreement with environmental temperature (Hertz, 1992). The real comparison is between the actual body temperature of the reptile and the body temperature that it would have if it did not thermoregulate. This can be determined by placing models of the lizards in their environment (Bakken & Gates, 1975) and by comparing their temperatures with those of the reptiles. The temperatures of the models are therefore indicators of the consequences of non-thermoregulation in the lizards.

METHODS AND MATERIALS

ANIMALS AND ENCLOSURE

Five *Physignathus cocincinus* (two males, three females) were observed for a total of 173.7 hours on selected days from autumn 1996 to summer 1998 dur-

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ing periods of cloudy weather, intermittent sunshine and hot sunshine. The lizards used in the study were adults (one male and one female) and subadults (one male and two females), with body masses ranging from 220 to 540 g. One subadult male lizard was mostly used for behaviour observations because of its timid nature. All others could be readily approached.

The observations were carried out in a tropical enclosure at the Taylor Hill Annexe of Huddersfield Technical College. This enclosure is subject to natural sunlight through glass, measures approximately 6 m by 5.5 m at ground level, and is 4 m high. The unit was divided horizontally into 20 equally-sized areas and vertically into four levels (i.e. ground + heights 1, 2 and 3). The main shade plant was Cyperus involucratus, although several other tall species (e.g. Ficus, Dracaena & Monstera) were also present. Enclosure diversity was enhanced by the use of tree branches and a small pond (2 x 1.5 m), including a waterfall. Artificial rainfall was also in operation. On cool, cloudy days the lizards were given the opportunity to elevate their body temperatures through the use of two 275 W spot lamps. One of these was placed above a tree branch at height 2, the other focused on a ground area. Both lamps were placed in areas frequented by the lizards.

BEHAVIOURAL OBSERVATIONS

The locations of lizards were recorded, together with their positions in relation to sunlight. Behavioural definitions are similar to those in Meek (1984, 1988a): basking, inactive and exposed to the sun's rays; shade, inactive in an area where no sunlight penetrated. Locomotory activity is self explanatory, but a further apparently important behaviour is partial basking. Here the animal sits with a mosaic of sunlit and shaded patches on its body. Behavioural observations were continuous and defined as the total number of minutes that each animal was involved in a particular activity. Entry into the water was also recorded

BODY TEMPERATURES

A total of 608 body temperatures were recorded, although these were actually measurements of skin surface temperatures recorded with an infrared detector (Digitron 232-3305 Pyrometer). This device is a noninvasive temperature indicator that can measure the infrared radiation emitted by reptiles (emissivity = 0.95, Tracy, 1982). Infrared energy is focused onto a thermophile via a germanium filter. The equipment has a resolution of 0.1° C, and the error is 1%, although the larger the lizard and the closer to the animal that the readings can be taken, the more accurate the measurement. Most animals used in the study allowed approach to within a few centimetres of skin surface without any apparent disturbance of behaviour patterns.

Estimates of the differences between skin surface temperature and cloacal temperature have been made for *Cyclura nubila* by Alberts & Grant (1997). For animals of 500 g body mass they found a relationship of the form:

$$(T_{b}-T_{ss}) = 0.776 (T_{ss}-T_{a})+T_{a}$$

where the estimated difference between cloacal temperature T_b and skin surface temperature T_{ss} is derived from air temperature T_a and a regression coefficient of 0.776. Examination of the data from Table 1 of their paper using least squares regression (including an additional measurement of a 6.65 kg *C. nubila* mentioned in their text) indicates that the observed differences were mass-based with the general error estimated as:

$$(T_{b}-T_{c})=0.00114x+1.1195, (R^{2}=0.7)$$

where body mass (x) is in kg. This equation predicts an overestimate of cloacal temperature of between 1.3 and 1.7°C in 200-500 g animals, equivalent to the size range of the lizards used in this study.

For comparison, measurements of the differences between skin surface and cloacal temperature were made on a 540 g adult male *P. cocincinus*. Cloacal temperatures were measured with a Yellow Springs Instruments Telethermometer (model 441A) and calibrated thermistor probe. The results indicated an increasing difference between skin surface and cloacal temperature with increasing temperature. The highest overestimate (n = 9) was 3.5°C (mean=2.2, SE=0.8). Mean underestimate (n=5) was 1.2°C±1.1 but in one instance reached 3°C. Generally the Alberts & Grant (1997) model for *Cyclura* would predict smaller errors.

LIZARD MODELS

To gather evidence of thermoregulation, models of P. cocincinus were constructed and placed in selected areas in the enclosure, usually in locations where the lizards had vantage points, but with one model placed in areas which they did not usually frequent (see Fig 1). The changes in temperature of the lizards and the models were compared using regression analysis, with the temperatures of the models treated as the independent variable and the temperatures of the lizards the dependent variable. The regression coefficients were then tested against the expected coefficients of a hypothetical thermoregulator (Bailey, 1981) which have expected values of 1.0 for thermoconformity and 0 for thermoregulation. This assumes a null hypothesis of no thermoregulation in the models (Bakken & Gates, 1975).

Lizard models were constructed of modelling clay with a light metal substructure. The models had similar mass and colour to the real lizards. A simple heating rate test comparing a 0.540 kg lizard and a 0.5 kg lizard model over a temperature range of 22.8 to 29.8°C showed that the lizard heated slightly more quickly than the model. The relationship is described by:

$$T_{h}=1.1T_{m}-1.17, (R^{2}=0.83)$$



FIG. 1. A series of histograms on a three dimensional plot showing the areas of the enclosure occupied by the lizards. The plots for individual lizards are: m_1 , a large adult male; m_2 , a subadult male; f_1 , a mature female; and f_2 and f_3 , subadult females.

where T_b is the temperature of the lizard, T_m model temperature and R^2 the coefficient of determination. This equation suggests that the models gave a reasonable estimate of potential lizard temperatures.

Lizard temperatures, model temperatures, air and substrate temperatures were recorded simultaneously. Air temperatures were taken 10 cm above the ground and substrate temperatures by directing the infrared pyrometer at the soil. Model temperatures were measured in a similar way to real lizard body temperatures, by pointing the pyrometer at the central area of either the animal's or model's mass. All body temperatures are shown with standard errors, and statistical tests are set at the 95% level using the F, t and Q-distributions (Parker, 1973; Bailey, 1981).

RESULTS

HORIZONTAL MOVEMENT

Fig. 1 is a series of histograms on a three dimensional plot showing the areas occupied by each lizard in the enclosure. The results are derived from the time the lizards occupied each grid square after the data were converted into percentages. No lizard during the observation periods was seen to move through all the grids (n=20), indicating a limited movement lifestyle. The large male (M_1) had been in the enclosure for the longest period (>2 years), but was not seen in five (25%) of the grids. The subadult male (M_2) had the most restricted movement, occupying only five grids and then mostly at higher areas in the canopy. The graphs show that each lizard occupied distinct areas with only small areas of overlap.



FIG. 2. Time spent by adults (left) and subadults (right) at different heights in the canopy. a, cloudy weather in winter; b, cloudy weather in summer; c, intermittent sunshine; d, hot sunshine. The data are shown as percentage frequencies from the highest (3) to lowest level (G).

TABLE 1. Summary statistics of the frequency of locomotor activity, the daily distances moved (in metres) during activity, and the frequency of entry into water (per hour) in *P.cocincinus* during different weather conditions. Data for *Activity* and *Entry into water* are shown as mean rates per hour; for distances travelled in each activity burst, the number of observations are shown. *n*=no. of days for *Activity* and *Entry into water*; *n*=no. of observations for *Distances travelled*.

	Range	Mean	SE	n
Activity:				
sunny weather	0.38-3.5	1.85	1.08	8
intermittent sunshine	0.32-3.8	1.25	0.99	10
cloudy weather	0-1.5	0.72	0.47	11
Distances travelled:				
sunny weather	0.1-5.0	1.02	1.01	73
intermittent sunshine	0.1-5.0	0.97	0.93	64
cloudy weather	0.1-4.5	1.05	0.96	56
Entry into water:				
sunny weather	0.43-0.87	0.58	0.17	4
intermittent sunshine	0.19-0.75	0.4	0.24	5
cloudy weather	0-0.45	0.17	0.15	6

VERTICAL MOVEMENT

Fig. 2 is a series of histograms showing the time different heights in the canopy were occupied by adult and subadult lizards during different seasons. On days with cool cloudy weather, either in summer or winter the lizards occupied higher levels of the canopy but responded to increasing amounts of sunshine by retreating to lower levels. However, subadults spent significantly more time at either height 3 (χ^2 =6367, df=3, P<0.001) or height 2 (χ^2 =14.82, df=3, P<0.01) than adults during any type of weather.

LOCOMOTOR ACTIVITY AND ENTRY INTO WATER

Table 1 shows the levels of activity and frequency of entry into the water. The data from all five lizards are pooled. Entry into the water is shown in both series of data as the mean rate of entry per hour. Activity and entry into the water increased proportionately with increasing levels of sunshine, being most frequent during hot sunny weather. During cloudy weather locomotor activity and entry into water did not occur at all on certain days. The attached standard errors show some variation within, and overlap between, the data sets. However, analysis of variance indicated that they were not homogenous; *activity*, $F_{2, 26}$ =3.50, P< 0.05; *swimming*, $F_{2, 12}$ = 4.32, P< 0.05 (*n*-2 days). Further analysis using a multiple range test with the Q-distribution (Parker, 1973) showed that the frequencies of activity and swimming observed under cloudy weather were significantly lower than recorded during the other weather periods (P < 0.05).

Distances travelled during bursts of activity during sunny weather, intermittent sunshine and cloudy weather, winter or summer, are shown in Table 1. Comparison of these data using analysis of variance showed that there were no significant differences between the data sets ($F_{2,191}=0.11$, P>0.05). Although activity there-



FIG. 3. Comparative behavioural patterns in *P. cocincinus* during: a, sunny weather; b, intermittent sunshine; c, cloudy weather in summer; d, cloudy weather in winter. The data are expressed as percentage frequencies of time in shade (S), partial basking (P/B), basking (B) and basking under a heat lamp (BL).

fore increased with increasing levels of sunshine, the actual distances travelled during each burst of activity did not.

THERMOREGULATORY BEHAVIOUR PATTERNS

Fig. 3 shows behaviour patterns for all five lizards during different weather and at different times of the year. The graph clearly shows major adjustments with different environmental conditions. For example, basking was only a major activity when the weather was cloudy. Shade seeking increased in proportion to the amount of solar energy available, being lowest through the winter period (7.5%), progressively increasing through cloudy weather in summer (17.1%) and intermittent sunshine (32.1%), and reaching a peak in hot sunny weather (54.5%). Partial basking was an important behavioural mechanism for controlling body temperature when the sun was shining or if there was intermittent sunshine (37% and 43.6% of observations respectively). In summer, when the weather was overcast, 76.4% of observations were of lizards situated in a full basking position and only 2.1% basking under a heat lamp. On overcast days in winter, 69.8% of lizard behaviour was spent in a full basking position, with a significant increase in basking under heat lamps (22.8%; χ^2 =19.7, P<0.001, using Yates' correction factor).

Although subadults spend more time at higher levels in the canopy than adults, the differences in time that either group spent in shade during hot sunshine (adults 56.4%, subadults 58.6%) or intermittent sunshine (adults 45%, subadults 41%) were not significant. There was also no significant difference in the amount of time that either group spent partially basking during hot sunshine (adults 37.1%; subadults 36.9%) or intermittent sunshine (adults 28%; subadults 30.5%). All χ^2 tests, *P*>0.05.

BODY TEMPERATURES

Fig. 4 shows the distribution of P. cocincinus body temperatures during different weather conditions, with the corresponding values for skewness and kurtosis; Table 2 shows the summary statistics. Body temperatures (data for all five lizards pooled) were higher when there was hot (mean=28.7±2.9°C, n=153) or intermittent sunshine (mean= 28.7 ± 2.3 °C, n=240) than on days (winter or summer) when the weather was overcast (mean=26.1±2.9°C, n=146). Comparison of these data using analysis of variance revealed a significant difference ($F_{2,537}$ =0.2, P<0.0001). Analysis with a multiple range test showed that cloudy weather data were significantly lower (P < 0.05). Body temperature distributions were negatively skewed during sunny weather and when the lizards used the basking lamps during overcast weather; under other conditions they showed positive skewness. The kurtosis value (k=3.9) exceeded that expected in a normal distribution (k=3.0) only during hot sunny weather. A rather low value (k=2.0) was found for data from overcast days when the lizards used basking lamps.



FIG. 4. Frequency distributions of body temperature of P. cocincinus at 2°C intervals during: a, sunny weather; b, intermittent sunshine; c, cloudy weather; d, cloudy weather when the lizards used heat lamps for basking. The data are shown as percentage frequencies, with the degrees of skewness (S) and kurtosis (K) for each histogram.

The data in Table 2 show that the interquartile ranges were narrower during hot sunny weather or intermittent sunshine than when the weather was overcast, both when the lizards used the heat lamps and when they did not. Statistical tests for differences between the body temperatures of adults and subadults were made in consideration of subadults spending more time at higher levels in the canopy. The differences (Table 2) were not significant. Presumably, subtle behavioural adjustments - e.g. postural changes or variations in the ratio of shaded areas to non-shaded areas on the body during partial basking - were employed to cancel out different thermal loads at different canopy heights.

Body temperature levels were significantly higher when the lizards used the basking lamps on cloudy days in winter (mean=31.0±4.0°C) than during cloudy days, either in summer (mean=26.9±2.9°C) or in winter (mean=25.7±2.6°C) when they did not; $F_{2, 213}$ =22.9, P<0.0001 and multiple range test (P<0.05).

TABLE 2. Summary statistics of body temperatures of *P. cocincinus* recorded under different environmental conditions. Q_1 and Q_3 represent the lower and upper quartiles. The mean values are given with one standard error, number of observation *n*, and minimum and maximum ranges. Statistical comparison of subadult and adult body temperatures, by analysis of variance, are also shown when appropriate.

	Mean	SE	п	Q ₁	Median	Q ₃	Max.	Min.
Sunny weather (sum	ner)							
Adults	28.4	3.01	90	26.7	28.3	30.7	34.7	19.0
Subadults	29.1	3.07	63	28.4	29.8	30.9	35.8	20.0
	$F_{1,151} =$	0.26, <i>P</i> >0	0.05					
Intermittent sunshine								
Adults	27.8	2.2	112	26.7	27.8	29.2	34.0	22.8
Subadults	28.4	2.4	128	27.0	28.2	30.7	34.8	22.2
	$F_{1,238} =$	3.096, <i>P</i> >	0.05					
Overcast (winter and	summer whe	n heat lan	nps were i	not used)				
Adults	26.2	3.1	98	23.8	25.8	28.8	33.4	20.0
Subadults	27.0	3.8	48	23.0	28.3	30.8	32.1	20.8
	F _{1,144} =	1.88, <i>P</i> >0	.05					
Overcast winter (hea	t lamps used)							
Adults	31.0	4.0	69	27.8	31.9	34.3	36.4	23.1

COMPARISON OF BODY TEMPERATURES WITH MODEL TEMPERATURES

During cloudy weather, regression analysis of the relationship between lizard body temperatures (T_b) and temperatures of the models (T_m) situated where the animals spent most of their time $(T_{m1}$ was taken at high elevations between levels 1 and 2, and T_{m2} was for ground areas) gave:

> $T_b = 0.72T_{m1} + 7.02, R^2 = 0.57, and$ $T_b = 0.91T_{m2} + 4.35, R^2 = 0.23.$

Data gathered during intermittent sunshine gave regression coefficients close to 0, but with very low coefficients of determination $(T_{m1}=0.18, R^2=0.11; T_{m2}=0.31, R^2=0.10)$ as did those for sunny weather $(T_{m1}=-0.04, R^2=0.08; T_{m2}=-0.03, R^2=0.07)$. These results indicated a number of trends. During

These results indicated a number of trends. During cloudy weather *P.cocincinus* thermoconformed with regression coefficients of 0.72 and 0.91 against the (non-thermoregulating) models. A series of *t*-tests (Bailey, 1981) confirm a significant departure from thermoregulation: $(T_{m1}) t=15.2$, P<0.001, df=179; $(T_{m2}) t=6.54$, P<0.001, df=179. Intermittent sunshine gave regression coefficients closer to those of a hypothetical perfect thermoregulator, but still significantly different values for *t*; $(T_{m1}) t=4.72$, $(T_{m2}) t=4.38$ - both P<0.001; df=174. The regressions differ significantly from thermoconformity (*t*-values 8.83 and 9.94, both P<0.001). Although just outside theoretical predictions, during hot, sunny weather with clear skies the

regression coefficients (-0.04, -0.03) were in agreement with thermoregulation (*t*-values -0.99 and -0.93, both P>0.05).

REGRESSIONS FOR NON-TERRITORIAL LOCATIONS

The regressions for body temperatures in relation to the temperatures of a model (T_{m3}) situated in areas which the lizards only rarely frequented gave similar results. For cloudy weather (not including data when the lizards used basking lamps) the results indicate thermoconformity, since:

$$T_{b}=1.02T_{m3}+0.93, R^{2}=0.51$$

with the regression coefficient not significantly different from 1.0 (P>0.05). There was no agreement between lizard body temperatures and model temperatures during hot or intermittent sunshine. The regressions were 0.08 (sunny weather, R^2 =0.008) and -0.08 (intermittent sunshine, R^2 =0.008).

BODY TEMPERATURES DURING CLOUDY WEATHER IN WINTER

Data gathered in the winter months was confined to days when the weather was overcast. On the days when *P. cocincinus* basked under the lamps, the regressions $(T_{m1}=0.05, R^2=0.11; T_{m2}=-0.06, R^2=0.05)$ showed very little association between body temperatures and model temperatures. The regressions do not differ significantly from 0 (*t*=0.21 and -0.15 respectively, both *P*>0.05).



FIG. 5. Thermal history of adult male (>500 g) *P. cocincinus* (solid squares), high-level model (T_{m1} , solid circles) and low-level model (T_{m2} , open circles) during a day of cloudy weather and occasional hazy sunshine. At the bottom of the graph are shown: the number of bursts of activity (solid triangles); time spent by the lizard in open areas (unhatched), partial shade (hatched), and in shade (solid); weather conditions as hatched areas for hazy sunshine and solid areas for cloudy weather.



FIG. 6. Thermal history of the lizard and models described in Fig. 5, but during a day with hot sunshine and continuously clear skies. The symbols on the main graph are those for Fig. 5. At the bottom of the graph are shown:the time the models spent in full sun (unhatched); filtered sun (hatched) and shade (solid). The circled points on the main graph indicate the readings of body temperature prior to entry into water.

BODY TEMPERATURES, MODEL TEMPERATURES, WEATHER AND TIME OF DAY

Fig. 5 shows an example of the thermal history of a large (alpha) male in relation to corresponding temperature changes of the models during cloudy weather with occasional hazy sunshine. In the lower part of the graph, the number of bursts of activity and the time the lizard spent in the sunshine, shade and partial shade are shown, in addition to weather conditions on the day (10 June). Mean lizard body temperature and SE for the period was $27.2\pm1.6^{\circ}$ C; compared to T_{ml} at $27.3\pm1.8^{\circ}$ C and T_{m2} , at $23.5\pm1.2^{\circ}$ C. The lizard spent 61% and 39% of its time at levels 1 and 2 respectively.

Models were placed at the junction of levels 1 and 2 (T_{m1}) and on the ground (T_{m2}) . Regressions of lizard body temperatures against model temperatures, with ttests against thermoconformity gave coefficients of 0.7 for T_{m1} (R²=0.70; t=0.19, P>0.05) and 0.76 for T_{m2} $(R^2=0.26; t=2.76, P<0.02)$. These results suggest that the lizard essentially thermoconformed. However, the behaviour of the lizard appears to show subtle thermoregulation using minimum activity. From 0800-1100 hrs the animal spent its time at height 2 in an open area close to the position of T_{mi}. During most of this period there was hazy sunshine, with about 45 mins of cloud when the lizard elevated its body temperature from 22-31°C. Two short bursts of activity at 1100 hrs (the only movement recorded throughout the observation period) taking it to a shaded area, resulted in stable body temperatures for the rest of the period. The brief period in hazy sunshine from 1215-1235 hrs was the result of the movement of the sun in relation to the stationary lizard.

Fig. 6 shows the thermal history of the same lizard during hot, sunny weather with clear skies (17 June). The lower part of the graph represents the amount of locomotory activity and the time the lizard or models spent in sunshine, partial shade or shade. The models were placed in similar locations to 10 June and the amount of time they were situated in sunshine, partial sunshine or shade was entirely dependent on the movement of the sun.

Compared to the data in Fig. 5, several important differences are apparent. There was an increased level of locomotory activity (eight bursts) and more entries into water (two). A large part of the day was spent lower in the canopy, with 47% at ground level and 53% at height 1. Entry into the water resulted in major reductions in body temperature. The temperature of the models reached lethal levels for lizards ($T_{ml} = 54.4$ °C; T_{m2} =45.2°C) and were high for most of the day. Lizard body temperatures, however, were in good agreement with body temperatures shown in Fig. 5 (intermittent sunshine and cloud, mean=27.2±1.6°C; hot sunshine, mean=26.04 \pm 2.54°C, $F_{1,53}$ =3.46, P>0.05). For a brief period the lizard was in full sun (2.2% of observations), the rest being spent either partially basking (17.9%) or in the shade (79.9%). The regression coefficients indicated no association with the models and no significant departures from 0 (T_{ml} =-0.09, T_{m2} =0.16; *t*-values -1.34 and 2.03, both *P*>0.05). The coefficient of determination was 0.06 for T_{ml} and 0.27 for T_{m2} . This shift in association between the models may reflect changes in vertical movement of the lizard in the canopy during different types of weather.

DISCUSSION

Continuous observation of *P. cocincinus* showed that by thermoregulating carefully, this species is active (i.e. able to remain abroad and vigilant) throughout even the hottest weather. Excessive heat loads are dealt with by microhabitat selection (e.g. by moving within the canopy or by entering water) and behavioural adjustments; primarily, partial basking and shade seeking. Under natural conditions it might have been concluded that at such times the lizards were inactive, as a result of the difficulties of observation.

However, it is possible that in a captive environment there were factors influencing the ability to thermoregulate and display natural behaviour patterns;. For example, the observations on different individuals were not independent. Social behaviour is one possible influencing factor. There are good reasons for believing that the effect was minimal in this study. Intense levels of social interaction were not apparent in the study animals, and in the species generally, may be confined to rivalry between large adult males, only one of which was present in the enclosure. These large males appear to be tolerant of subadults and females, who interact with one another only at a very low level, if at all (nothing of any significance was observed during the study). It was not uncommon for lizards to be situated close to each other, and the subadult male was even observed in the close vicinity of the large male.

The data in Fig. 1 show not only limited home ranges, but also territory overlap between certain animals. It is likely that this limited movement lifestyle is based on energy costs rather than constraints on social behaviour - the greater range of the adult female possibly being related to exploratory behaviour for nest sites. The presence of subadults (Fig. 2) higher in the canopy may be an adaptation to avoiding terrestrial predators, larger animals finding it more difficult to perch on the thinner vegetation. The absence of statistical departures in body temperature levels between adults and subadults suggests a limited thermoregulatory cost in this behaviour, if any at all.

An open-mouthed posture - similar in appearance to gaping - was regularly observed in the adult male and female *P. cocincinus* during the study period. Simultaneous measurements of body temperatures (range 23-31°C, n=12) and observations of behaviour suggest that the function is probably (in conjunction with headbobbing and arm waving) for communication between individuals rather than a thermoregulation mechanism. This may have been a communication between the two

adults since they were in the vicinity of one another at such times - although the open-mouthed posture was never observed simultaneously in the two animals. Furthermore, there was no intense follow up, such as evidence of chasing, after it occurred.

Activity patterns of reptiles in tropical environments are constrained by high heat loads (e.g. Hailey & Coulson, 1996) rather than by the primary requirement of basking to raise body temperature in temperate forms (e.g. Avery, 1976; Avery et al., 1982), particularly when body masses approach 1 kg (e.g. Meek, 1988a). The results of this study are in good agreement with those of Stevenson (1985), who estimated that habitat use and adjustments in activity were the most effective mechanisms for a 1 kg reptile to control body temperature, a view supported in subsequent studies of a tropical tortoise Kinixys spekii by Hailey & Coulson (1996). The behaviour of P. cocincinus fits theoretical predictions on the effects of high heat loads on sit-andwait predators, which should increase activity as a result of solar radiation falling on selected vantage points. Sit-and-wait predation is energetically economical, since four- to six-fold increases in locomotory activity increase energy costs by approximately 30-50% in reptiles (Huey & Pianka, 1981).

Sentinel behaviour and site location in sit-and-wait predators are crucial for detecting predators and prey, and in males for monitoring the movements of females and rival males. Thermoregulatory behaviour in an ecological context must be sensitive to these requirements if they are to be balanced optimally. *P. cocincinus* were present for long periods at regular perch sites, often with no major locomotory activity for several days. However, they were always alert and presumably scanning the surrounding area. Partial basking is a behavioural mechanism that contributes in an important way to balancing heat losses and gains. This permits the retention of scanning opportunities by avoiding a full retreat into dense shade.

In a tropical environment, the costs of basking and raising body temperature for P. cocincinus should be low, as movements between sunlit and shaded areas will be short. When conditions allow, thermoconformity saves energy by reducing shuttling, but any movement away from cover increases the costs of shade seeking (Asplund, 1974; Lee, 1980; Huey, 1982). Precise thermoregulation during hot sunshine, and thermoconformity during overcast weather, may reduce the energy costs of thermoregulation in tropical reptiles (e.g. Lee, 1980; van Marken Lichtenbelt et al., 1997). For example, in shaded forests Anolis lizards thermoconform, but they thermoregulate carefully in open habitats (Huey, 1974; Lister, 1976; Lee, 1980; Huey, 1982). Thermoregulatory costs may be reduced in semi-aquatic reptiles which thermoconform when water temperatures are high but bask when the weather is cooler or overcast (Edgren & Edgren, 1955; Meek, 1983). Increases in body mass further increase the costs of shuttling, although how important this is in *P. cocincinus* is not clear. It is interesting to note, however, that short term shuttling is not abandoned in *Crocodylus porosus* and *Crocodylus johnstoni* until body masses of 20 kg are exceeded (Grigg *et al.*, 1998).

The ability to feed at relatively low body temperatures (minimum observed, based on five cool days: 21.8°C, mean=22.6±0.53) and the occasional use of basking lamps during prolonged periods of overcast weather, could suggest an adaptation to cooler conditions during wet seasons. Why P. cocincinus should use basking lamps to raise body temperature only on certain days is not immediately obvious, although under natural conditions irregular basking has been observed in free-living reptiles, including temperate forms (e.g. Auth, 1975). The low kurtosis value shown in Fig. 2 may be relevant. It could, for example, indicate a reluctance to temporarily abandon sentinel locations; movement to the sites of the basking lamps may be energetically or ecologically costly and any benefits gained from raised body temperatures must be outweighed by the costs incurred. There was no evidence of daily shuttling between basking and perch sites, that is there was invariably only one visit to a heat lamp on such days.

Even allowing for the differences between skin surface temperature and core temperature, the body temperature ranges recorded in P. cocincinus were lower than the body temperatures recorded from the Australian form P. longirostris (Licht et al., 1966). P. longirostris (Licht et al., 1966) and P. lesueurii (personal observation) occupy more open and structurally simpler habitats than P. cocincinus, and may have greater thermoregulatory costs (Asplund, 1974; Huey, 1982). Relatively low body temperatures in P. cocincinus could also indicate a wide safety margin before critically high temperatures are experienced, as has been indicated in Kinixys spekii (Hailey & Coulson 1996). Even temperate reptiles of this size range are in danger of overheating during hot weather (Meek, 1984), particularly when involved in crucial activities (Meek, 1988b). Low body temperatures also reduce metabolic costs (Christian et al., 1984) and when inactivity and cryptic colouration are the main predatory/ anti-predator mechanisms, sprint speed - an ecologically important performance trait in lizards (Garland & Losos, 1994) - may not be so critical.

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SQUAMATE RELATIONSHIPS BASED ON C-MOS NUCLEAR DNA SEQUENCES

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Relationships among squamate families have classically been difficult to establish, with morphological characters being interpreted to give many different topologies. Here we combine new *C-mos* nuclear DNA sequence data with those already published to assess relationships of 19 families within the Squamata. Monophyly of all the families examined is upheld. Many relationships between families are estimated, although it appears there may have been rapid cladogenesis associated with the origins of the Squamata.

Key words: Phylogeny, squamates, C-mos

INTRODUCTION

Squamate relationships have remained contentious since Camp's (1923) "Classification of the Lizards". Despite extensive analyses based on morphological characters many relationships remain unknown. Most widely accepted are the relationships suggested by Estes et al. (1988), although the analysis has been criticized (Kluge, 1989), and alternative suggestions for relationships have been made using different morphological characters (Presch, 1988). Surprisingly, the advent of DNA sequence data has had little impact on our understanding of squamate relationships. Although many studies have examined inter-familial relationships (e.g. Hedges et al., 1991; Harris et al., 1998), these have been limited due to their use of mitochondrial DNA sequences, which are typically saturated before the divergence times necessary to estimate relationships across squamates.

Recently Saint *et al.* (1998) used a fragment of the nuclear gene C-mos to investigate relationships of Australian reptiles relative to their overseas relatives. They showed that C-mos was likely to be a single copy gene in squamates, had no introns, and that a fragment of about 400 base pairs could be amplified across many squamate families. Graybeal (1994) had already shown that C-mos might be phylogenetically informative among taxa that had diverged up to 400 mya. To estimate relationships across squamates, we have extended the number of families included, and compared the estimates of phylogeny produced from Maximum Parsimony (MP) and Maximum Likelihood (ML) with those previously derived from morphological characters.

METHODS

The additional species examined were: F. Cordylidae: Cordylus cordylus; F. Gekkonidae: Bunopus tuberculatus, Stenodactylus doriae; F. Iguanidae: Dipsosaurus dorsalis, Iguana iguana; F. Lacertidae: Acanthodactylus scutellatus, Lacerta kulzeri, Podarcis hispanica; F. Trogonophidae; Diplometophon zarudnyi; F. Xantusidae: Lepidophyma gaigae, Xantusia vigilis. These were selected to cover five families not included by Saint et al. (1998), and to extend the number of the family Gekkonidae examined from one to three.

Total genomic DNA was extracted from small (1 or 2 mm³) pieces of tail tissue. The material was finely diced and digested with proteinase K (Kocher *et al.*, 1989). Purification was by phenol/chloroform extractions (Sambrook *et al.*, 1989), followed by centrifugal dialysis through a Centricon 30000 MW membrane (Amicon). Polymerase Chain Reaction (PCR) primers used in both the amplification and the sequencing were G73 and G74 (Saint *et al.*, 1998). PCR conditions were the same as those used by Saint *et al.* (1998). Successful PCR products were purified using a Qiaex II kit (Qiagen), and sequenced from both strands on an Applied Biosystems DNA Sequencing System.

SEQUENCE ANALYSIS

Genbank accession numbers are AF148702 to AF148712. The sequences were aligned by eye to the previously published sequences (Genbank AF039462 to AF039482) of Saint *et al.* (1998). The aligned sequences were 375bp long. The codon reading frame was infered by comparison with the published sequences. Of the new sequences, all the lacertids and the two geckos had a deletion of seven codons, and the *Diplometophon* had an eight codon deletion. These were in the same region (bp 727-768 of human C-mos

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FIG. 1. Single most parsimonious tree derived from an analysis of C-mos nucleotide sequence. Numbers above branches indicate bootstrap support (1000 replicates). Numbers below branches indicate boostrap support from an MP analysis based on the amino acid sequence, with all changes weighted equally. See text for details. The tree was rooted using the *Crocodylus, Chelodina* and *Elseya* sequences.

sequence), and overlapped deletions also found in the skink *Lipinia noctua* and the teiid *Cnemidophorus tigris*. They were therefore treated as missing data in the analyses.

The data were analysed using PAUP* (Swofford, 1998). When estimating phylogenetic relationships among sequences, one assumes a model of evolution regardless of the optimality criteria employed. Determining which model to use given the data is a statistical problem (Goldman, 1993). We used the approach outlined by Huelsenbeck & Crandall (1997) to test alternative models of evolution, employing PAUP* and Modeltest (Posada & Crandall, 1998). A starting tree was obtained using neighbour-joining. With this tree, likelihood scores were calculated for various models of evolution and then compared statistically using a chisquare test with degrees of freedom equal to the difference in free parameters between the models being tested. The null hypotheses tested in this way included: (1) nucleotide frequencies are equal; (2) transition rates are equal to transversion rates; (3) transition rates are equal and transversion rates are equal; (4) rate homogeneity exists within the data set; and (5) there is no significant proportion of invariable sites. Once a model of evolution was chosen, it was used to estimate a tree using maximum likelihood (Felsenstein, 1981), using random sequence addition and a heuristic search with 10 replicates. Also an MP analysis was performed. Two hundred and nine of the 375 characters were parsimony-informative. A 10 replicate heuristic search was carried out, and support for nodes was estimated using the bootstrap (Felsenstein, 1985) technique, with 1000 replicates. A further MP analysis was carried out on the translated amino acid sequences. All changes were weighted equally.

RESULTS

Using MP, 209 of the 375 characters were parsimony-informative. A 10 replicate heuristic search found one MP tree with 892 steps. (CI= 0.46, HI= 0.54). Support for nodes was estimated using the bootstrap (Felsenstein, 1985) technique, with 1000 replicates (Fig. 1). In the translated amino acid sequence, 60 characters were informative. A ten replicate heuris-

TABLE 1. Tests of hypotheses relating to the model of evolution appropriate for phylogeny reconstruction (Huelsenbeck and Crandall, 1997). *P*-values were obtained using the computer program Modeltest (Posada & Crandall, 1998). Due to the performance of multiple tests, the significance level of rejection of the null hypothesis should be adjusted via the Bonferroni correction to $\alpha = 0.01$.

Null Hypothesis	Models Compared	-lnL _o	-lnL ₁	df	Р	
Equal base frequencies	H ₀ : JC69, H ₁ : F81	5010.1	5005.5	3	0.012	
Equal ti/tv rates	H ₀ : JC69, H ₁ : K80	5010.1	4763.8	1	< 0.001	
Equal ti and equal tv rates	H ₀ : K80, H ₁ : GTR	4763.8	4761.6	3	0.340	
Equal rates among sites	H ₀ : K80, H ₁ : K80+G	4763.8	4582.3	1	< 0.001	
Proportion of invariable sites	$H_0: K80+G, H_1: K80+G+invar$	4582.3	4568.9	1	< 0.001	
Molecular clock	H ₀ : no rate heterogeneity,	4635.8	4568.9	34	< 0.001	
	H ₁ : rate heterogeneity					



FIG. 2. Single maximum likelihood tree, derived using the K80 model with estimation of the proportion of invariant sites and a discreet approximation of the gamma distribution. See text for details.

tic search found three equally parsimonious trees of 376 steps (CI=0.61, HI=0.39). Support for nodes was again estimated using 1000 bootstrap replicates (Fig. 1). With ML, using Modeltest (Posada & Crandall, 1998) we concluded that the Kimura 80 model (transition/ transversion ratio = 2.6584), with a gamma distributed rate heterogeneity model (a= 3.0825), and an estimated proportion of invariable sites (0.2905) was the most appropriate model of evolution for these data. The data did not fit a molecular clock (Table 1). A ten replicate heuristic search using random sequence addition with this model produced a single maximum likelihood tree of score -ln 4568.9 (Fig. 2).

DISCUSSION

Analysis of our extended data set supports many of the conclusions drawn by Saint *et al.* (1998). The analyses based on *C-mos* sequences support the monophyly of the squamates, and that the closest living relative is *Sphenodon punctatus*. Within the squamates, all the superfamilies and families where multiple species were sampled came out as monophyletic groups - Agamidae (99% bootstrap support from MP tree), Amphisbaenia (100%), Booidea (99%), Gekkonidae (95%), Iguanidae (100%), Lacertidae (100%), Pygopodidae (95%), Scincidae (99%) and Xantusidae (100%). In the analy-

ses of the nucleotide sequences, the teiid Cnemidophorus tigris comes out basal to all other squamates, although this is not the case when the amino acid sequence is analysed. Based on morphological characters, teiids are usually regarded as the sister taxa to lacertids (e.g. Estes et al., 1988). The basal position in this analysis could be due to the presence of a paralogous sequence in teiids, or it could be due to an artifact in the data such as long branch attractions (Felsenstein, 1978), or due to massive convergence in the morphological characters. Long branch attraction could be due to rate variation or inadequate sampling. Taxon sampling should not be a problem, as we have included C-mos sequences of lacertids, which are thought to be closely related to teiids (Estes et al., 1988). Rate variation cannot be ruled out, as the data do not fit a molecular clock (Table 1), and it is clear from the ML analysis (Fig. 2) that Cnemidophorus has the longest external branch of all the squamates sampled. Since its position is only weakly supported (55% bootstrap in MP tree), and since the branches immediately above its position are extremely short, it cannot be placed with much confidence by this data set.

Based on morphological characters, the Scincomorpha is thought to include Scincidae, Cordylidae, Xantusidae, Lacertidae, Teiidae and Gymnophthalmidae (Estes et al., 1988), with some authors suggesting that the amphisbaenians should be included (e.g. Schwenk, 1988). Excluding Gymnopthalmidae, which was not sampled, and Cnemidophorus tigris, these taxa are also associated by the MP analysis, with the Xantusidae being the sister taxon to the Scincidae, and with the next closest relative being the Cordylidae. These are the same relationships suggested by Presch (1988) based on morphological characters. The two amphisbaenians included, Bipes biporus and Diplometophon zarudnyi, are strongly grouped as monophyletic (100%), and appear to be the sister taxa to the Lacertidae. Evidence from amphisbaenian fossils also suggests they may be members of the Scincomorpha (Wu et al., 1996). Within the Lacertidae, the monophyly of the subfamily Lacertinae - Lacerta kulzeri and Podarcis hispanica (Harris et al., 1998) is weakly supported in the MP analysis.

The two Iguanids included, *Iguana iguana* and *Dipsosaurus dorsalis*, are strongly associated with the phrynosomatid *Sceloporus grammicus*, and this is also supported by morphology (Estes *et al.*, 1988). Most closely related to these is the clade made up of the Agamidae and Chamaeleonidae, again something found using morphological characters (Estes *et al.*, 1988). Monophyly of the two anguimorph families Anguidae and Varanidae was recoved by both ML and MP, but with low bootstrap support (66%).

Saint *et al.* (1998) labelled the subfamily Diplodactylinae (*Carphodactylus* and *Strophorus*) as members of the Gekkonidae. Kluge (1987) included the Diplodactylinae in the Pygopodidae because of a shared derived character of the muscle encircling the external ear opening. C-mos sequences support this, with the Diplodactylinae being sister group to the Pygopodidae (*Delma*) in both the ML and MP analysis (95% bootstrap support).

One difference between the MP and ML analyses was in the placement of *Eublepharis macularius*. ML analyses associate it with the Gekkonidae, while the MP analysis places it as sister taxon to the Pygopodidae. Morphological characters suggest that it is basal to a clade of these two groups (Grismer, 1988). While C-mos sequences clearly group Gekkonidae with Pygopodidae and Eublepharidae, the exact relationship between these three groups remains unresolved.

Most of the other intra-familial relationships are extremely weakly supported, as shown by very short internal branches in the ML analysis. As suggested by Saint *et al.* (1998), this could be the result of rapid cladogenesis, or simply a result of the limitations of using only one gene region to examine relationships. Only the inclusion of more sequence data will help to resolve this, although it is clear that *C-mos* is an extremely useful gene for examining many aspects of squamate relationships.

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A MULTIVARIATE APPROACH TO THE SYSTEMATICS OF ITALIAN RAT SNAKES OF THE *ELAPHE LONGISSIMA* COMPLEX (REPTILIA, COLUBRIDAE): REVALIDATION OF CAMERANO'S *CALLOPELTIS LONGISSIMUS* VAR. *LINEATA*

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We used multivariate analysis to study patterns of geographic variation in morphology in the Aesculapian snake, Elaphe longissima, in Italy and other parts of its range, in order to evaluate the status of the southern Italian form, hitherto known as E. I. romana. Although that taxon was previously regarded as weakly differentiated, a recent study based on blood proteins showed a high level of differentiation, similar to that observed between full species of European Elaphe. Fourteen characters relating to external morphology were recorded from 104 adult specimens of Elaphe longissima from 52 localities, and subjected to multivariate analysis. The results show that southern Italian specimens are clearly distinct from those of other populations. The morphological divergence is coupled with a parapatric distribution pattern and a sharp transition at the contact zone in central Italy. We found the distribution limits of the two taxa to be situated about 100 km further south than previously suggested. Parapatry among morphologically clearly distinct forms, and differences in blood plasma suggesting considerable evolutionary divergence, provide reasons for considering the southern Italian form as a separate evolutionary species from E. longissima. The name Coluber romanus Suckow, 1798 is not available for the southern Italian species, for which the oldest available name is Callopeltis longissimus var. lineata Camerano, 1891, in the combination Elaphe lineata.

Key words: Elaphe, multivariate analysis, taxonomy, morphology, zoogeography

INTRODUCTION

The Aesculapian snake, *Elaphe longissima* (LAURENTI, 1768) (Type locality (restr.): Vienna, Austria) is the most widespread rat snake in Europe. It occurs in the Mediterranean and submediterranean regions, from northern Spain to the Middle East. Due to its wide distribution, it has been an intensively studied species (see Böhme, 1993). However, basic aspects of its intraspecific classification and pattern of geographic variation remain uncertain or poorly defined. This applies particularly to the Italian populations, which are the subject of this study.

A recent study of blood plasma proteins in European *Elaphe* species (Lenk & Joger 1994) noted that specimens from Sicily currently assigned to *Elaphe longissima romana* (Suckow, 1798) are strikingly distinct from other European *E. longissima* populations, possibly at a higher taxonomic level than hitherto believed.

Mertens & Müller (1928) first recognized the distinct nature of the southern Italian taxon, and applied the name *Coluber romanus* Suckow, 1798 to this form. They regarded it as a subspecies of *Elaphe longissima* and restricted the range from 'warmer parts of Europe, Spain, France, Italy, and especially surroundings of Rome' (Suckow, 1798) to 'central and southern Italy, Sicily' with the type locality 'surroundings of Rome', but without any explicit analysis.

Capocaccia (1964) conducted the first substantial study of the subdivision of *E. longissima* in Italy. Her description of *romana* (grey belly; the shape of the temporal blotches, which are united with the posterior submandibular blotches; a high number of ventral scales; and a rather light coloration) deviates considerably from Suckow's, which was chiefly based on the occurrence of longitudinal stripes on the posterior part of the trunk. Further doubts on the accuracy of the type locality and the availability of the name *romana* Suckow (1798) for the southern Italian form arose from Cattaneo's (1975) description of a melanistic population of *E. l. longissima* from the Roman littoral.

The prevailing uncertainty regarding the status of the southern Italian Aesculapian snake gave us reason to initiate the following study. Here, we consider some newly defined characters, as well as those used by Capocaccia (1964), in order to re-analyse the distinctness of both forms in Italy and additional localities in the Balkans by means of multivariate analysis of morphological characters. Multivariate techniques, and especially ordination methods such as principal components analysis (PCA), have been shown to be useful tools in revealing patterns of geographic variation in the generalized phenotype which are often obscured when characters are analysed individually (e.g. Thorpe, 1976, 1980; Wüster, Otsuka, Thorpe & Malhotra, 1992, Wüster, Warrell, Cox, Jintakune & Nabhitabhata, 1997).

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MATERIALS AND METHODS

Twenty-eight characters of external morphology and colour pattern were recorded from 104 adult specimens from the Italian Peninsula and other parts of the species range. A special effort was made to include specimens from the Balkans, some of which share characters with southern Italian populations *sensu* Capocaccia (1964) (see above).

In addition to the 104 specimens mentioned above, eight juvenile specimens were examined, but not included in the further analyses, as ontogenetic variation in some colour pattern characters would have affected the results of the analysis. Furthermore, one adult specimen (LPPC 313 from Udine / Northern Italy) was excluded from further analyses due to highly aberrant scalation characters.

In order to identify characters useful for multivariate analysis, a two-way analysis of variance (BMDP 2V -Dixon, 1991) was performed to determine which characters show significant geographic variation between localities, sexual dimorphism, or both. For this purpose, specimens were grouped into six geographic test groups on the basis of collecting gaps, potential physiographic barriers, and past taxonomic subdivisions. Only characters showing significant geographical variation were subjected to multivariate analysis. Most of them were qualitative, and coded in binary form (0,1). Progressive states (e.g. absent, inconspicuous, conspicuous) were coded accordingly (e.g. 1, 2, 3).

Characters found to be invariable, unrecordable in a large percentage of specimens, or incompatible with the assumptions of PCA were not included in further analyses. However, some of these characters do convey considerable information and will be discussed below.

PCA was used to reveal the pattern of morphological variation in the complex, with the specific aim of testing whether the southern Italian populations and the remaining populations form two homogeneous groups that are distinct from each other. PCA was run on a computer program written by R. G. Davies (Imperial College London) and modified by R. S. Thorpe (University of Wales, Bangor). All variables were standardized to zero mean and unit standard deviation. To rule out confusion due to sexual dimorphism, both sexes were analysed separately. The analyses were run on the data from individual specimens, to avoid a priori assumptions of taxon membership. For all runs, the eigenvector coefficients of each character for the first principal component were extracted to identify the characters that contribute most to the observed pattern of variation.

RESULTS

The results of the ANOVA reveal that 13 characters show significant geographical variation among males and 14 among females (Table 1). These characters were subjected to an initial PCA (PCA 1) as described above. TABLE 1. Characters used in the PCA. Characters 7-10 were recorded as follows: Starting with the last ventral we moved 10, 20, 30, etc. scales forwards and transversely counted the dorsal scale rows at each site. The relative positions of the segments where the changes occured were assessed.

- 1. No of ventrals (Arnold & Burton, 1979).
- 2. No of pairs of subcaudal scales.
- 3. Ventral colour: % ventral scale position of the transition from bright to dark ventrals.
- 4. Dorsal background colour: micro-speckling on undamaged dorsal scales at posterior trunk predominantly (see. Fig 5) due to absent epidermal pigmentation.
- 5. Presence of longitudinal stripes at midbody.
- 6. Temporal blotches fused with posterior mandibular blotch at least on one side.
- 7. % ventral scale position of reduction from 19 to 17 dorsal scale rows.
- 8. % ventral scale position of reduction from 21 to 19 dorsal scale rows.
- 9. % ventral scale position of reduction from 23 to 21 dorsal scale rows.
- 10. % ventral scale position of addition from 21 to 23 dorsal scale rows.
- 11. Bright blotches, bright blotches with greyish spots on each scale, or absent nuchal blotches, (at least above the elongation of the mouth slit).
- 12. Keeling of dorsals of posterior body absent (0), present in less than 50% of scales (1), present in more than 50% (2).
- 13. White dashes at midbody absent, present in less than 10 % of scales, present in more than 10%.
- 14. Presence of greyish marbling on the bright venter.

In the males, this analysis resulted in two well-defined geographical groups separated along the first principal component: one group comprises specimens from southern Italy, and the other comprises specimens from northern Italy and the remainder of the range of the *E. longissima* complex (Fig. 1). In females, however, the resolution was less clear, as the first principal component scores of some specimens from the Balkans lay within the range of southern Italian specimens. Reassessment of these Balkan specimens showed them to be melanistic individuals.

Some characters (3 and 11, Table 1) recorded from all specimens were affected by melanism, so that character states recorded as identical in melanistic Balkans specimens and non-melanistic southern Italian specimens may not have been homologous. Consequently, the PCA was repeated, with the exclusion of the suspect characters. This re-analysis (PCA 2) resulted in a good resolution in the females, as both geographical groups were separated along the first principal component (Fig. 2). In the males, a similar pattern was exhibited, as the first PC separates southern Italian specimens from the others. However, two non-melanistic individuals,



FIG. 1. PCA 1 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factors, of females (a) and males (b), extracted from all characters. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 37.97% and 13.24% of the total variance in males, and 36.88% and 12.93% in females.

one from Castelfusano (Rome) and one from the Balkans, grouped with the southern Italian form, which was not the case in the PCA using all characters.

A third analysis (PCA 3) was run on all specimens from the Italian Peninsula and Sicily. As none of the Italian specimens was melanistic, homologies of character states in characters 3 and 1 l were unproblematic, and consequently, all characters were included. In both sexes, this resulted in a clear separation of northern and southern specimens along the first principal component (Fig. 3).

Loadings of eigenvectors reveal that, in both sexes, numbers of ventrals, dorsal colour, nuchal blotches, and positions of dorsal scale row reductions appear to be the most important characters in distinguishing the two forms (Table 2). However, the remaining characters also contribute more or less strongly to the separation of the two groups.

In order to relate the separation of the two morphological groups to their distribution, the first principal component scores of all specimens were plotted against their geographical position along a latitudinal transect along the Italian Peninsula. Fig. 4 shows a striking morphological shift between northern and southern Italian specimens, with no indication of clinal variation. Specimens of the nominate form can be found south to



FIG. 2. PCA 2 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factors, of females (a) and males (b), extracted from all but characters 3 and 11. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 34.27 % and 15.06 % of the total variance in males and 31.38 % and 14.96 % in females.

around 41 ° N, but are replaced by the southern Italian form to the south of that latitude.

ADDITIONAL CHARACTERS

Although the frequency of specimens with dark longitudinal stripes is not related to locality, the shape of the stripes, when present, is highly diagnostic. In specimens from southern Italy, the stripes are narrower than the light spaces between them. They are set off rather clearly by the light background colour on the posterior body. In specimens from the remainder of the range, the stripes are broader than the spaces between them. It is clear that the two morphs follow different genetic programs.

DISCUSSION

Our data suggest a marked differentiation between northern and southern Italian specimens of the *Elaphe longissima* complex. This is evident from the PCA plots, which clearly separate the two forms along the first principal component. The characters listed as diagnostic for the southern Italian form by Capocaccia (1964) can also be found in occasional specimens from other parts of Europe. If only Capocaccia's characters are used, there is a risk of misidentifying some specimens from northern Italy as belonging to the southern

TABLE 2. Normalized eigenvector coefficients of the first principal components of PCA 1 (all characters all specimens), PCA 2 (all characters, exclusively 3 and 11, all specimens), and PCA 3 (all characters, Italian specimens only) in all females and males.

_	Characters	PCA 1 female	PCA 1 male	PCA 2 female	PCA 2 male	PCA 3 female	PCA 3 male
1	No of ventrals	0.294	0.318	0.332	0.335	0.319	0.245
2	No of subcaudals	-0.051	-0.221	-0.080	-0.247	-0.078	-0.155
3	Ventral transition bright/dark	0.389	0.358	- -	2 4 0	0.375	0.394
4	Dorsal colour	0.393	0.333	0.447	0.397	0.377	0.399
5	Presence of stripes	0.006	-0.155	0.022	-0.202	0.010	0.022
6	Temporal blotches	0.202	0.265	0.227	0.289	0.250	0.174
7	Pos. red. 19-17	-0.245	-0.296	-0.297	-0.354	-0.280	-0.261
8	Pos. red. 21-19	-0.387	-0.337	-0.459	-0.412	-0.367	-0.404
9	Pos. red. 23-21	-0.302	-0.254	-0.390	-0.333	-0.292	-0.330
10	Pos. add. 21-23	0.168	0.169	0.195	0.246	0.166	0.051
11	Bright nuchal blotches	0.393	0.391	-	-	0.367	0.395
12	Keeling	-0.073	-0.226	-0.105	-0.242	-0.035	-0.119
13	White dashes	-0.171	-0.133	-0.260	-0.142	-0.156	-0.240
14	Marbling	-0.214	-	-0.251	-	-0.244	-
	Percentage of total variance	36.88	37.97	31.38	34.27	45.45	44.56





FIG. 3. PCA 3 - scatter diagram of the component scores (normalized vectors) of the first and second PCA factor of Italian females (a) and males (b) extracted from all characters. Squares indicate *E. l. longissima* individuals, circles indicate individuals of the southern Italian form. The first two principal components account, respectively, for 44.56% and 11.38% of the total variance in males and 45.45% and 15.73% in females.

FIG. 4. Scatter diagram of the 1st. PC scores of PCA 3 of females (a) and males (b) on a transect running along the Apennine Peninsula. Sardinia excluded.

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Italian form (but not vice versa). This is especially true for specimens from the province of Lazio. However, if Capocaccia's characters are redefined and simultaneously analysed with additional characters, a clear pattern is obtained that separates two geographically distinct groups of snakes. This is also true for those individuals of the nominate form that show superficial similarities with the southern Italian form.

A problem arises with non-homologous character states in the case of melanistic specimens. In particular, melanistic specimens from the Balkans exhibit convergent similarities with the southern Italian form in some characters. However, if characters affected by melanism are left out, the remaining characters clearly assign them to the nominate form, although the overall differentiation is somewhat reduced (Fig. 2). When melanistic specimens are excluded, maximum resolution is gained when all characters are used, as is shown for the Italian samples (Fig. 3). These analyses demonstrate a clear differentiation, with no overlap between northern and southern specimens along the first principal component. Variation within each group is due to individual differences, which can be of considerable magnitude, as indicated by outliers in both directions (Fig. 3). These rare specimens are responsible for the lack of any absolute diagnostic character for the two forms.

Some non-melanistic specimens from the southern part of the Balkans also show superficial similarities with the southern Italian form (e.g. grey venter, high ventral scale counts). However, our PCA results indicate that these can be unambiguously assigned to the nominate form (Fig. 2).

To test for the occurrence of intergrades, first principal component scores of Italian specimens were plotted against their origin along a latitudinal transect along the Italian Peninsula (Fig. 4). This approach showed no evidence of intergradation between the two taxa near their contact zone. Furthermore, variation within the two forms is not associated with geographical latitude. Thus, considerable variation exists within each group, but is clearly distinct from the variation representing the evolutionary signal of the two independent evolutionary lineages.

The contact zone between the two forms is located further south than hitherto believed, at least in western Italy. Whereas Capocaccia (1964) assigned all populations from the coastal regions of Lazio to the southern Italian form, all our specimens from the surroundings of Rome and Circeo (50 km S-E of Rome) are clearly assignable to the nominate form, as noted by Cattaneo (1975). This is also the case with additional material, such as published illustrations (Bruno & Maugeri, 1990) and juvenile snakes not included in the analysis (MRSN R1317, from Cocullo, province Aquila, and BM 1914.4108, from Lake Albano).The contact zone identified in this study is situated more than 100 km south of that mentioned by Capocaccia (1964). The transition between the two forms is abrupt, and occurs over a distance of less than 100 km between Rosello (Abruzzo - northern form) and Benevento (Campania - southern form; see Fig. 7). Although our comparatively coarse sampling scale does not allow the determination of the exact position and nature of the presumed contact zone, our data suggest that a sharp transition is far more likely than a clinal variation over hundreds of kilometres. Furthermore, Capocaccia (1964) mentioned specimens from the Matese region that clearly belong to the northern Italian form. As Capocaccia's description of the nominate form is reliable, the contact zone appears to lie within the 50 km separating the Matese mountains and Benevento.

STATUS OF THE SOUTHERN ITALIAN FORM

Our morphological results, coupled with the molecular results of Lenk & Joger (1994), strongly support the hypothesis that the southern Italian form represents a separate taxon from the nominate form. The southern Italian form and the nominate form represent two clearly distinct, but reasonably homogeneous, entities.

Morphological differences on their own do not necessarily indicate that two distinct groups of populations represent separate evolutionary lineages: morphological variation may simply represent an adaptation to some ecological parameter, and thus reflect selection pressure due to current ecological conditions rather than separate ancestry (e.g. Thorpe, Brown, Malhotra & Wüster, 1991, Thorpe, Brown, Day, Malhotra, McGregor & Wüster, 1994, Thorpe, Malhotra, Black, Daltry & Wüster, 1995). However, this is unlikely to be the case in Elaphe longissima: parallel patterns of variation in plasma proteins suggest a separate history for the southern Italian form and the nominate form. In addition, the nominate form is relatively homogeneous across a very largerange, which spans a wide variety of climatic and environmental conditions, and the contact zone between the southern Italian and nominate forms does not correspond to any sharp environmental transition.

There remains the problem of the status of the southern form, and, in particular, the question of whether it represents a full species or a subspecies of E. *longissima*. Our data provided no evidence for sympatry or hybridization between the two forms. There is no physiographic barrier likely to separate the two forms, and a distribution gap that divides both ranges into two allopatric entities is unlikely. If a hybrid zone exists, our results suggest that it is relatively narrow, with a maximum width less than 100 km, and probably less than 50 km. The distributions of the two species can thus be described as approximately parapatric.

Parapatric contact and hybrid zones typically occur between closely related taxa, such as sister species or subspecies of one species. Although hybridization may occur in nature in those cases, any selection against hybrids results in reduced fitness of the offspring (Barton FIG. 5. Character states 'dark dorsals' and 'bright dorsals' are defined on the basis of whether fine speckling is visible on the entire scale (right) or largely invisible due to strong epidermal pigmentation (left).

& Hewitt, 1989). Thus selection acts against the free diffusion of genes between both taxa and helps maintain distinctness and independent evolution in different lineages.

We assume a similar scenario for the two Italian taxa. If no genetic break existed, a greater degree of morphological intergradation should be observed. Taking into account that, on grounds of zoogeographic and climatic constraints, both taxa are likely to have occurred together in Italy for at least several thousand years (or far longer), the parapatric distribution suggests a considerable extent of genetic incompatibility. This is consistent with the results of Lenk & Joger (1994): the level of divergence in plasma protein patterns between the two forms is similar to that typically found between uncontested full *Elaphe* species.

Based on this evidence, the southern Italian and the nominate form appear to be distinct lineages on different evolutionary trajectories. In recent years, the general trend has been to recognize such lineages as separate evolutionary or phylogenetic species (Cracraft, 1989; Frost & Hillis, 1990; Wiley, 1981). In view of our results, we regard the southern Italian form as a separate evolutionary species, distinct from *E*. *longissima*. Alternatively, some may prefer the superspecies/semispecies terminology of Mayr (1969), which may also be applicable in this case: in this, the southern Italian form would be a semi-species within the *E. longissima* superspecies.

Further studies should be focused on the exact determination of the contact zone in middle Italy, and could reveal interesting insights into the extent and maintenance of stable contact zones. Therefore surveys should be intensified in the corresponding regions, preferably in conjunction with a population genetic study.

NOMENCLATURE

The question of the correct name for the southern Italian species is complex, particularly due to two major problems associated with the name *romana* traditionally used for it. First, the restricted type locality of *romana* ('vicinity of Rome' - Mertens & Müller, 1928) lies within the range of the nominate form.

Second, Suckow's description of *romana* (1798) which is nearly identical with that of Lacépède's Aesculapian snake (Lacépède, 1789), is incompatible with the appearance of the southern Italian species or any related taxon. He mentioned a reddish brown body colour, a dark longitudinal stripe at each side, darkening to black along its ventral edge, black margins on white scales next to the ventrals forming a lateral line of white triangles, head rather thick, 239 (175 ventrals and 64 pairs of subcaudals) black and white spotted scales, and a length of 3.5 - 4.5 feet (105-150 cm). In the absence of a type specimen to confirm the identity of Suckow's intended taxon, the name *Coluber romanus* Suckow, 1789 must be regarded as unavailable for the southern Italian species.

The earliest description that agrees with our findings is that of Camerano (1891), who studied a specimen from Naples and named it *Callopeltis longissimus* var. *lineata*. He described it as having a grey belly, and four



FIG. 6. Dorsal and ventral side of the holotype of *Elaphe lineata* (Camerano, 1891) MZUT 942 (original number MZUT 434) from the type locality Naples.

Males		E. longis	sima (n = 3	35)		E. lineata	n (n = 23)	
Characters (no.)	mean	SD	max.	min.	mean	SD	max.	min.
length head-trunk [cm]	87.44	12.39	111.00	75.00	81.76	18.22	105.00	55.00
length tail [cm]	22.87	3.24	28.00	15.00	20.91	6.03	34.00	10.00
ventrals (1)	225.77	4.01	236.00	214.00	232.13	3.44	238.00	225.00
subcaudals (2)	82.91	3.35	94.00	78.00	79.30	2.60	82.00	72.00
pos. red. 19-17 (7)	82.67	8.74	69.00	100.00	91.68	4.20	86.85	100.00
pos. red. 21-19 (8)	62.16	2.83	56.14	68.47	67.09	2.77	61.38	71.21
pos. red. 23-21 (9)	55.59	2.37	50.90	60.53	58.03	2.33	53.19	61.54
pos. red. 21 -23 (10)	25.33	5.08	17.03	42.99	22.60	4.71	16.67	35.62
Females		E. longis	sima (n = 3	31)		E. lineate	a (n = 16)	
Characters (no.)	mean	SD	max.	min.	mean	SD	max.	min.
length head-trunk [cm]	73.52	11.23	90.00	48.00	70.28	13.30	83.50	49.00
length tail [cm]	16.69	2.62	22.00	12.00	16.25	3.60	20.00	10.00
ventrals (1)	225.06	4.54	235.00	214.00	231.88	2.58	235.00	225.00
subcaudals (2)	72.52	3.41	79.00	65.00	71.81	4.12	80.00	61.00
pos. red. 19-17 (7)	93.96	7.99	69.16	100.00	99.47	2.14	91.46	100.00
pos. red. 21-19 (8)	64.61	3.47	59.83	74.03	73.87	4.57	65.82	82.98
pos. red. 23-21 (9)	57.66	4.25	38.33	63.20	60.98	2.76	56.90	65.67

TABLE 3. The quantitative description of characters in the two species. For explanation of characters see also Table 1.

dark brown, longitudinal stripes running along the back and the flanks from the head to the tail. This description identifies his specimen as belonging to the southern Italian species. Although Camerano did not explicitly designate a type specimen, we assume that his type is identical with MZUT 942 (formerly MZUT 434) of the Museo di Zoologia e Anatomia Comparata Torino, which originates from Naples and has a note 'det. Camerano'. We also examined a second specimen mentioned in the same publication (Camerano, 1891), from Serra San Bruno (Calabria) (MZUT 935) in that collection. Consequently, *Callopeltis longissimus* var. *lineata* Camerano, 1891, in the combination *Elaphe* *lineata*, is the oldest available name for the southern Italian form, and MZUT 942 represents its holotype.

ELAPHE LINEATA (CAMERANO, 1891) NEW COMB.

Holotype. MZUT 942 (original number: MZUT 434; Fig. 6).

Terra typica. Naples.

Diagnosis. A European rat snake that differs from the most similar species, *E. longissima*, in usually having a light dorsal colour, grey ventral colour, often four dark longitudinal stripes that are narrower than the spaces between them (broader in *longissima*), in high average counts of ventral scales, slightly smaller size than

20 10	E. long	gissima	E. lineata		
Character states (no.)	females $(n = 31)$	males $(n = 35)$	females $(n = 16)$	males $(n=23)$	
bright nuchal blotches (11)	89	85	5	0	
keeling (12)	16	60	8	37	
white dashes (13)	66	65	40	50	
venter with marbling (14)	35	0	-	-	
ventral transition bright/dark (3)	13	18	94	100	
bright dorsal background (4)	0	20	100	91	
presence of stripes (5)	32	17	38	48	
temporal blotches (6)	39	26	94	91	

TABLE 4. The frequency [%] of qualitative character states in both species.



FIG. 7. Locations of *E. longissima* and *E. lineata* in central Italy mentioned in the text (solid squares).

Elaphe longissima, and in not having bright nuchal blotches (see Table 3 and 4).

Elaphe lineata attains at least 102 cm total body length in females, and 139 cm in males. It is likely that *E. lineata* may considerably exceed this length, as is known *for E. longissima* (Böhme, 1993; Schulz, 1995).

Except for one individual from Modica, Sicily (MZUT 940), nuchal blotches are always absent in E. *lineata*. In *E. longissima*, the blotches (yellow to orange in males, ivory to yellow in females) are usually apparent, except in strongly melanistic individuals. This character is not subject to ontogenetic variation: both juvenile snakes and adults of *E. longissima* exhibit nuchal blotches, whereas juveniles and adults of *E. lineata* do not.

The background dorsal body colour is usually rather light, showing a fine speckling on the scales (Fig. 5). The dorsum often carries four dark longitudinal stripes, which are narrower than the spaces between them on the posterior part of the body. The white dashes on individual dorsal scales, known from *E. longissima*, are less developed in *E. lineata* and usually restricted to the dark stripes. In *E. longissima*, the dorsal ground colour is comparatively dark, especially on the posterior part of the body (Fig. 5). Striped specimens are also frequent in *E. longissima*.

The ventral colour of E. lineata is yellowish under the head and neck, and usually uniformly grey under the trunk and tail. Light spots on the lateral edges of the ventrals mark a line that is running along the whole trunk. Only one female from Modica, Sicily, mentioned above, exhibits a totally light venter. In contrast, the ventral colour of E. longissima is uniformly yellow, especially in males after ecdysis. The ventral colour of females is pale yellow. Some specimens (particularly females) exhibit grey spots or marbling on the posterior part of the venter, but light colours always predominate on each scale. Predominantly dark ventral scales are very rare. Some specimens from the Caucasus region lack yellow colours but show merged dark and light marbling on their venter in equal proportions on each scale. In melanistic specimens from the Balkans, the posterior two-thirds of the venter is covered by dark

grey pigmentation. However, a few small bright spots of the size of a dorsal scale usually remain visible.

In Capocaccia (1964), the shape of the dark temporal and mandibular blotches is used for discriminating between the two forms. In our specimens from southern Italy, the temporal blotches are usually connected with the posterior submandibular blotches, whereas in *E. longissima* these blotches are usually separated. However, we found that a notable number of *E. longissima* specimens depart from that rule (Table 4).

The number of ventrals in *E. lineata* (mean=232) exceeds that of *E. longissima* (mean=225; see Table 4). The dorsal scale row reduction from 21 to 19 rows is displaced caudally in *E. lineata* compared with *E. longissima*. In males the reduction is located at a ventral scale position corresponding to 67% (mean) of the trunk in *E. lineata* and 62% (mean) in the nominate form. In females, the corresponding values are 73 % (mean) in the southern Italian form and 65 % (mean) in the nominate form.

Finally, living *E. lineata* have a reddish iris (Schulz, 1995), whereas the iris of *E. longissima* is brown or greyish in life. In our personal observations, a red iris colour was never seen in living specimens of the nominate form, $(n \ge 200)$, but present in all *E. lineata* (n=20).

Juvenile specimens of *E. lineata* can be recognised by the lack of bright nuchal blotches, the reddish iris, and often a marked dark pattern on the pileus. Dark spots are always present on the dorsal parts of the trunk in hatchlings but often disappear during early youth. On the neck, they are fused into several larger spots.

While many characters showed geographic variation, and in particular differentiation between *E. longissima* and *E. lineata*, other characters were chiefly affected by sexual dimorphism, with parallel trends in both species. This was manifested in the relative length of the tail, the number of subcaudals, number of dorsal scale rows, ventral colour (see above) and the extent of keeling (see Tables 3 and 4).

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APPENDIX

The specimens of this study were provided by the following collections:

MTKD Staatliches Museum für Tierkunde, Dresden, MZUF Museo Zoologico de La Specola, Firenze, MZUT Museo Zoologico dell' Università di Torino, MRSN Museo Regionale di Scienze Naturali, Torino, SMF Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, ZIN Zoological Institute, Russian Academy of Sciences, St. Petersburg, ZFMK Zoologisches Museum und Forschungsinstitut Alexander Koenig, Bonn, BM Museum Natural History. London, LLPC Luiselli, Luca, private collection, LPPC Lenk, Peter, private collection, EONAR Exhibition Oasi Naturale WWF Abetina di Rosello

Elaphe longissima

Adult specimens: Albania -Illyria (BM 1920.I.20.466) Bosnia -Herzegovina, Capoljina (BM 96.9.5.10) -Travnik (BM 93.II.13.I) Bulgaria -?

(MTKD 6177, MTKD 3350, MTKD 15656) -Bistrica (MTKD 28560) -Sandanski-Bistrica, Liljanova (MTKD 25440, MTKD 28285) -Pirin mountains Bansko (MTKD 25491) -Baile Herculane (MTKD 28808, MTKD 29108, MTKD 29107, MTKD 26622, MTKD 28807) -Arkutino (MTKD 27316, MTKD 19535, MTKD 25493, MTKD 19386) -Harmanli (MTKD 6802, MTKD 7114, MTKD 4699, MTKD 4135, MTKD 4698) -Primorsko (MTKD 8855) Georgia-Abchasia, Pizunda (ZIN 17070, ZIN 11847, ZIN 17068, ZIN 17069) - Novyy Afon (ZIN 12964) Greece -Vernon, Vitsi (MZUF 30862) -Mt. Olympus (BM 1931.12.15.2) Italy -Trentino, Caldaro (SMF 70693) -Trentino, Primolano (SMF 70478) -Trentino, Jenesien (SMF 18431, SMF 18432) -Friuli, Udine (LPPC 313) -Lombardia, Mori (MTKD 44259) -Liguria, Albenga/ Allassio (ZFMK 23111) -Liguria, Laigueglia (ZFMK 58155) -Emilia-Romagna, Bologna (RR.1964.1558) -Toscana, Viterbo, Farnese (MZUF 15647) -Umbria, Perugia, Mt. S. Maria Tiberina (MZUF 7960) - Umbria, Perugia, Collazone (MZUF 26601) -Lazio, 10 km south of Roma (LLPC), -Lazio, Monte Circeo, Commune della Marsione (2 x LLPC) -Lazio, Roma, Via Ada (LLPC) -Lazio, Castelfusano (LLPC) -Lazio, 15 km north of Veio (LLPC) - Abruzzo, Pacentro (LPPC 312) -Abruzzo, Rosello (EONAR) Yugoslavia -? (MTKD 3146) Romania-?(MTKD 32629, MTKD 32626, MTKD 32628, MTKD 32627) Russia -Sochi (ZIN 15303, ZIN 11608) Switzerland -Ticino, Mte. Brè, Locarno (SMF 44131) - Ticino, Arcegno (SMF 23989) -Ticino, Maggia (SMF 52979, SMF 59280, SMF 48243)

Juveniles: Italy Lazio, Lago Albano (BM 1974.4108), -Aquila, Cocullo (MRSN R1317), Toscana, Campagnatico (MZUF 55992)

Elaphe lineata

Adult specimens: Italy -Campania, Benevento, Pannarano (MRSN 1362) - Campania, Napoli (ZFMK 5950, ZFMK 23112, ZFMK 5949, ZFMK 5948, MZUT 942) - Campania, Roccarainola (MZUF33017, MZUF 33019, MZUF33005, MZUF33002, MZUF 33018, MZUF 33007, MZUF 32991, MZUF 33016, LPPC295) Campania, Vesuvio (MZUF 33020, MZUF 33021) -Campania, Sarno, Pianta Marina (MZUF32992, MZUF32993) -Campania, Salerno, Laurino (MRSN 1269) - Campania, Salerno Giffoni Valle Piana (MRSN 1422) -Basilicata, Potenza, Viggiano (MZUF 31666) -Basilicata, Potenza, Cazzavella (MZUF 18733) -Calabria, Jozzo, Cardinale (MZUF 31206, MZUF 30859, MZUF 30698, MZUF 31960, MZUF 30699, MZUF30529) -(MZUF 30087) -Calabria, Mellara (MZUF 22498) - Calabria, Delianuova (MZUF 35175) -Calabria, Serra San Bruno (MZUT 935) -Sardegna, Santu Lussurgiu (SMF 65083) - Sicilia, Modica (MZUT 940) -Sicilia, Dioppo Monreale (ZFMK 16557) -Sicilia, Acireale (SMF36390) - Sicilia, Agrigento (SMF 47100) - Sicilia, Palermo (SMF 54226) - S-Italy (ZFMK 52264)

Juveniles: Italy Calabria, Novalba di Cardinale (MZUF 30087), -Campania, Avella (MZUF 32995), -Campania, Roccarainola (MZUF 33003, MZUF 33004), Calabria, Delianuova (MZUF 31176).

FEEDING HABITS OF SYMPATRIC DISCOGLOSSUS MONTALENTII, DISCOGLOSSUS SARDUS AND EUPROCTUS MONTANUS DURING THE BREEDING SEASON

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The diets of three Corsican amphibians, *Discoglossus montalentii*, *Discoglossus sardus* and *Euproctus montanus*, were studied in the Ospedale region during the breeding season. Adult specimens were collected in or around breeding pools and were stomach flushed in the field. Prey taxa included a large variety of terrestrial and aquatic prey items of variable size, indicating opportunistic predation. All species were able to catch their prey both on land and in water, but varied in the proportions of aquatic and terrestrial prey consumed. *E. montanus* fed largely upon benthic macroinvertebrates, suggesting predation in deep water; *D. sardus* mainly captured terrestrial prey; and *D. montalentii* showed a mixed feeding strategy, preying upon both terrestrial and aquatic prey categories in similar proportions. *Discoglossus sardus* showed the highest standardized value of niche breadth ($D_s = 0.769$), compared to *D. montalentii* and *E. montanus* ($D_s = 0.544$ and $D_s = 0.523$ respectively). When prey size frequency distributions were compared, no specific differences were observed. These results indicated that, at least during the breeding season, trophic segregation among sympatric amphibians was maintained by different foraging strategies, and that the three species exploited contiguous microhabitats in different ways.

Key words: Corsica, Discoglossus, Euproctus, foraging strategy, sympatry

INTRODUCTION

On the Mediterranean island of Corsica seven amphibian species, including three endemic forms (Euproctus montanus, Discoglossus montalentii and Salamandra corsica), are known to occurr (Delaugerre & Cheylan, 1992; Gasc et al., 1997). The Corsican painted frog D. montalentii was the last species to have been described on the basis of biochemical and morphological characters (Lanza et al., 1984), and -due to its confusion with the congeneric Tyrrhenian painted frog D. sardus - little is known about its biology and ecology. Discoglossus montalentii is mainly a mountain species living in pristine or semi-pristine forest streams, while D. sardus breeds from sea level to about 1300 m, in a variety of aquatic habitat types (Clark & Lanza, 1990; Lanza, Vanni & Brizzi, 1992a,b). The altitudinal distribution of the two species overlaps broadly, and in several areas they are found together (Clarke & Lanza, 1990; Salvidio et al., unpublished data). During a two year field study on the distribution and ecology of D. montalentii and D. sardus, we collected data on their feeding habits together with those of the sympatric Corsican brook salamander E. montanus. This gave us the opportunity to analyse their feeding behaviours and to compare their foraging modes in order to investigate whether they were partitioning food resources when they coexist.

STUDY AREA AND METHODS

Samples were collected in the Ospedale forests (Southern Corsica, 20 km NW of Portovecchio) from April to June, both in 1996 and 1997. The study area, a *Pinus laricio* forest ranging from 700 to 1000 m a.s.l., was located between the villages of Ospedale and Zonza, which are separated by a linear distance of about 12 km. Although very similar morphologically, *D. montalentii* and *D. sardus* were identified in the field mainly on the basis of their hindlimb length, which is relatively greater in *D. montalentii* (Clarke & Lanza, 1990; Salvidio *et al.*, unpublished data); in some cases, field identification was confirmed by dissection and by chromosomal analysis (Gaetano Odierna *in litteris*).

With the exception of Salamandra corsica (see below), all amphibians were captured in or near (i.e. less than 1 metre from) water. All specimens were measured from the tip of the snout to the posterior end of the vent (SVL) to the nearest mm, and toe clipped at first capture; thus, stomach contents were analyzed only once for each individual. Diet composition was obtained by stomach flushing (Fraser, 1976; Joly, 1987), and stomach contents were preserved in the field in 70% ethanol. Stomach flushing was performed at least twice on each specimen and, if no prey was obtained, the stomach was considered empty. Where possible, food items were identified to order or family, and measured to the nearest 0.1 mm under a dissecting microscope. Aquatic invertebrates were classified using Sansoni's (1988) identification keys of freshwater

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macroinvertebrates. Prey volume (V) was calculated by considering each item as a cylinder or a sphere, and was expressed in mm³. Additionally, prey items were classified as aquatic or terrestrial, with a third category comprising prey that dwell on land, in shallow water or mud (e.g. metamorphosing anurans, mites, dipteran larvae, earthworms), as well as unidentified prey items.

Simple correspondence analysis (CA), based on a contingency table with predators as rows and prey taxa as columns, was used to obtain a graphical representation of the association between species and prey categories. In this analysis, a small sample of the Corsican fire salamander S. corsica (four specimens collected in the Ospedale region, and one from Haute Asco in Northern Corsica, all of them captured in terrestrial habitats) was included for comparison, as this species feeds exclusively upon terrestrial invertebrates (Kuzmin, 1994). The Corsican fire salamander sample was used in the CA as a supplementary row element; thus, it was represented in the Euclidean space determined by the first two axes, but did not contribute to the calculation of the other species location (Greenacre, 1993). Correspondence analysis was performed using Minitab 11.12 statistical software (Minitab Inc., 1996). Prey volume frequency distributions were compared with a Kolmogorov-Smirnov two sample test (Siegel & Castellan, 1988). The standardized version of Simpson's diversity index $D_s = (D - 1)/(N - 1)$, in which D is the Simpson's index $(D = 1/S p_i^2)$ and N is the number of prey taxa categories, was used to estimate trophic niche breadth. This standardized index ranges from 0, when only one resource category is exploited, to 1 when all categories are exploited with the same frequency (Barbault, 1981).

RESULTS

During this study, 144 amphibians were stomach flushed (Table 1). The proportion of stomachs containing at least one prey item (inorganic and plant matter were excluded from data analyses) ranged from 100% in *S. corsica* to 63% *in D. sardus*, and a chi-square analysis, based on the three largest samples (i.e. excluding the *S. corsica* sample), indicated that there were no differences in the frequency of fasting animals ($\chi^2 =$ 4.4, df=2; *P*=0.108).

The Euproctus montanus sample consisted of sexually mature individuals: 46 males and 6 females. The sample of *D. sardus* comprised 23 males, 6 females and 2 subadults, and that of *D. montalentii* 2 males, 13 females and 4 subadults. All subadult individuals were excluded from the quantitative data analysis. As the frequencies of terrestrial and aquatic prey categories did not differ significantly between sexes [*E. montanus* $\chi^2=0.2$, df=1; *D. sardus* $\chi^2=3.4$, df=1; *D. montalentii* χ^2 =1.8, df=1; *P*>0.05 in all cases, all tests with Yate's correction for continuity (Siegel & Castellan, 1988)], food data from males and females were combined for interspecific comparisons.

Table 2 gives the complete data set of the diet composition by taxonomic group and by volume. In the Ospedale forests, amphibians consumed a wide range of terrestrial and aquatic invertebrates. The Corsican brook salamander was the only species that preyed upon vertebrates: one unidentified urodelan larva and one postmetamorphic froglet (Discoglossus sp.) were found in the stomachs of two males, and a third male swallowed four eggs belonging to its own species. The distribution of prey items according to their life-style indicated that all species were able to feed both in aquatic and terrestrial environments; however, E. montanus fed mainly on benthic macroinvertebrates, in particular Plecoptera, Ephemeroptera and Tricoptera larvae, while D. sardus primarily consumed terrestrial prey (Isopoda and Araneida). D. montalentii captured both freshwater, and terrestrial food items in similar proportions. These results suggest that the brook salamander was foraging mainly in deep water, while the two discoglossids probably preferred surface or shallow water. The frequencies of prey according to their life-style (aquatic vs terrestrial) were overall significantly different (χ^2 =31.3, df=4, P<0.001), but when species were compared pairwise, the Corsican painted frog and the Corsican brook salamander showed similar feeding habits: D.montalentii - E. montanus, $\chi^2 = 4.9$, df=2; P=0.088; D. montalentii - D. sardus, $\chi^2 = 9.6$, $df=2, P=0.008; D. sardus - E. montanus, \chi^2=30.7, df=2,$ P < 0.001. The utilization of taxonomic categories by volume showed similar findings (Table 2), as D. montalentii and E. montanus were the species depending the most upon aquatic food resources (58% and 30% of the total ingested volume, respectively). The standardized Simpson index D_{s} calculated on the basis of taxonomic categories, indicated that D. sardus had the widest trophic niche breadth ($D_s = 0.769$), E. montanus the narrowest ($D_s = 0.523$), and D. montalentii an intermediate value ($D_s = 0.544$); but when prey volumes were considered, all species showed similar - and relatively narrow values - of niche breadths ($0.299 < D_s < 0.356$; see Table 2).

The results of the CA based on the complete data set are shown in Fig. 1. The first and second axes explained 38% and 24% of the total variance, respectively. In this plot, both *Discoglossus sardus* and *S. corsica* were projected in the bottom right quadrant of the plot, in strict association with their terrestrial prey categories. *Discoglossus montalentii* and *E. montanus* were projected in different quadrants and their diets were characterized by swimming invertebrates (Dytiscidae and Heteroptera) and by amphibian eggs or froglets, repectively.

The analysis of prey utilization by size showed that in all species small food items ($V < 9 \text{ mm}^3$) contributed a large proportion (more than 50 %) of the total number of prey, while relatively large items tended to be rather infrequent. When prey-size distributions (Fig. 2) were compared, these amphibian populations showed similar

TABLE 1. Number of stomach-flushed specimens and mean snout-vent length (SVL) of adult *D. montalentii*, *D. sardus*, *E. monta*nus and *S. corsica* containing at least one prey item in the stomach.

Species	Sample size N	Sample with prey	Adult sample with prey SVL (mm)±SD		
D. montalentii	22	19 (86%)	44.4 \pm 9.57 (<i>N</i> = 15)		
D. sardus	49	31 (63%)	48.4 ± 6.27 (<i>N</i> = 29)		
E. montanus	68	52 (76%)	$49.6 \pm 3.69 (N = 52)$		
S. corsica	5	5 (100%)	$96.6 \pm 18.04 (N = 5)$		

TABLE 2. Food composition by taxa (N) and by volume (V) expressed in mm³, and standardized trophic niche breadth (D_i) of D. montalentii, D. sardus, E. montanus, and S. corsica.

		D. mo	ontalentii	D. sa	rdus	E. mon	ıtanus	<i>S. cc</i>	orsica
	Prey type		(15)	(29))	(52	2)	(5)
		N (%)	V (%)	N (%)	V (%)	N (%)	V (%)	N (%)	V (%)
	Aquatic								
1	Coleoptera Dytiscidae (adults)	2(5)	149(13)	-	-	-	-	-	-
2	Coleoptera (adults)-Dytiscidae excluded	6(15)	47(4)	5(8)	28(2)	2(3)	5(0)	-	-
3	Coleoptera (larvae)-Dytiscidae excluded	-	-	4 (6)	64 (4)	3 (4)	10(0)	-	-
4	Trichoptera (larvae)	4(10)	367(32)	3(5)	116(7)	15(20)	328(13)	-	-
5	Ephemeroptera (larvae)	1(2)	25(2)	-	-	12(16)	170(7)	-	-
6	Plecoptera (larvae)	2(5)	13(1)	-	-	8(11)	28(1)	-	
7	Heteroptera (adults)	3(7)	37(3)	2(3)	28(2)	1(1)	15(1)	-	-
8	Heteroptera (larvae)	2(5)	14(1)	-	-	-	-	-	-
9	Vertebrata (eggs, larvae)	-	-	-	-	5(6)	192(8)	-	-
	Subtotal	20(50)	653(58)	14(22)	236(14)	46(61)	748(30)	-	-
	Terrestrial								
10	Isopoda	-	-	3(15)	68(4)	2(3)	16(1)	-	-
11	Myriapoda	2(5)	32(3)	6(9)	67(4)	-	-	2(17)	18(0)
12	Pseudoscorpionida	-	-	-	-	1(1)	0(0)	-	-
13	Araneida	4(10)	35(3)	7(10)	73(4)	4(5)	11(0)	2(17)	13(0)
14	Gastropoda Pulmonata	1(2)	87(8)	6(9)	550(33)	2(3)	515(21)	6(50)	2694(99)
15	Collembola	-	-	2(3)	3 (0)	-	-	-	
16	Diptera (adults)	1(2)	6(0)	5(8)	29 (2)	1(1)	0(0)	-	-
17	Coleoptera (adults)	2(4)	9(1)	2(3)	24(1)	-	-	1(8)	1(0)
18	Hymenoptera Formicidae	2(5)	16(1)	3(5)	12(0)	-	-	-	-
19	Hymenoptera Formicidae excluded	1(2)	2(0)	-	-	-	-	-	-
20	Lepidoptera (larvae)	-	-	1(2)	14(0)	-	-	-	-
	Subtotal	12(30)	186(16)	35(54)	840(51)	10(13)	542(22)	11(92)	2726(99)
	Land, shallow water or mud-dwelling								
21	Acarina	-	-	-	-	1(1)	0(0)	-	-
22	Oligochaeta	2(5)	28(2)	6(9)	230(14)	4(5)	86(4)	-	-
23	Diptera (larvae)	1(2)	251(22)	8(12)	334(20)	8(11)	858(35)	1(8)	23(1)
24	Invertebrata unidentified	5(13)	22(2)	2(3)	14(0)	6(8)	9(0)	-	-
25	Vertebrata (Discoglossus froglets)	-	-	-	-	1(1)	203(8)	-	-
-	Subtotal	8(20)	301(26)	16(25)	578(35)	20(26)	1147(47)	1(8)	23(1)
	Total	40	1140	65	1654	76	2437	12	2749
	Standardized niche breadth D_s	0.544	0.300	0.769	0.299	0.523	0.356	-	-



FIG. 1. Simple Correspondence Analysis plot showing the association between the diets of sympatric *D. montalentii*, *D. sardus*, *E. montanus* and their food categories (numbers correspond to prey categories in TABLE 2). *S. corsica* was added as a supplementary row element (see text).



FIG. 2. Prey size distributions in sympatric *D. montalentii*, *D. sardus* and *E. montanus* from the Ospedale forest.

patterns of food utilization (Kolmogorov-Smirnov two sample test, *P*>0.10 for all comparisons).

DISCUSSION

Discoglossus montalentii, D. sardus, and E. montanus were collected in or near aquatic habitats, where they were mating and spawning. At this time all three species should have been exposed to the same array of prey items. This may not be true, for example, in summer, when E. montanus adopts completely terrestrial habits (Goux, 1953; Michelot, 1992) and D. montalentii is still active in or near aquatic habitats (Salvidio et al., unpublished data). The study species foraged on a wide variety of prey, suggesting a generalist foraging strategy. The presence in their diet of freshwater prey items indicated that they were all capable of swallowing their food under water. Thus, these findings confirm that Discoglossus are adapted to take prey underwater (Boulenger, 1897-1898) as newts do, a rather unusual behaviour in comparison to all other Eu-

ropean anurans (except for the discoglossid Bombina), which feed primarly on land, even during their aquatic breeding season (Chiminello & Generani, 1992; Nöllert & Nöllert, 1995). When prey size distributions were analysed, there were no statistically significant differences in prey utilisation between predators, despite their variation in body size (see Table 1). On the other hand, important taxonomic prey differences appeared when foraging behaviours were analysed. Euproctus montanus ingested mainly benthic hydrobionts, showing a clear tendency to feed in deep water. This foraging mode appeared qualitatively similar to that of the congener Pyrenean brook salamander E. asper (Montori, 1997). Conversely, D. sardus foraged mainly on land, while D. montalentii showed a mixed feeding strategy capturing prey both in terrestrial and aquatic microhabitats in similar proportions. These findings are most likely due to increased time spent foraging in different microhabitats by each species. Thus, these observations suggest more aquatic foraging behaviour for the Corsican painted frog, compared to the congeneric Tyrrhenian painted frog, at least during their reproductive period.

Prey type, and especially prey size, have been considered to play an important part in resource partitioning in amphibian assemblages (Toft, 1985). Differences in food size were usually related to the body size of the predator, while differences in prey type were attributable to habitat preferences, or to the time in which food is available (Pilorge, 1982). In the present case, amphibians were sampled in the same habitat, and thus the observed differences in food habits could be related to species-specific trophic behaviour. On the basis of these observations, these coexisting species seemed to partition food resources by adopting different foraging strategies, as contiguous microhabitats were exploited in different ways by the three species.

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SPATIAL STRUCTURE AND REGULATION OF A POPULATION OF THE BROWN FROG RANA MACROCNEMIS IN GEORGIA

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A spatially structured population of the brown frog *Rana macrocnemis* was studied over seven consecutive years in the valley of a small mountain river. Frogs spawned in numerous temporary pools and puddles situated along the river bed. The overall number of reproductive females varied between 868 and 1146 during the course of study. The population had a 'source-sink' spatial structure: 88% of all froglets metamorphosed in just a few of the sites. Density-dependent mortality of larvae did not significantly affect the overall number of metamorphs. However, the variation in size of the whole population and of one of the two sub-populations was density-dependent. The most important cause of regulation appears to be density-dependent dispersal of juveniles from sources to adjacent sinks. Sinks often had a high number of 'traps' – large well-illuminated pools that frogs prefer for egg deposition but which desiccate during the course of larval development more often than the deep and cold, less attractive breeding sites.

Key words: Rana macrocnemis, frog populations, spatial ecology

INTRODUCTION

The concept of spatially heterogeneous populations is an important component of population ecology theory (Hanski, 1996). Two related models are most popular: the 'classic' metapopulation model (Hanski & Gilpin, 1991) and the 'sources and sinks' model (Pulliam, 1988, 1996; Levin, 1989; Howe, Davis & Mosca, 1991). The latter model assumes that a habitat can be subdivided into two parts - sources with a positive reproductive balance, and sinks, that are sustained by migration from sources. Amphibians are favourite subjects for metapopulation studies because their populations can often be subdivided into spatial groupings attached to separate spawning sites. The spatial structure of some amphibian populations can be described in terms of the 'sources-sinks' model (Gill, 1978; Ishchenko, 1979; Sinsch, 1992; Toxopeus et al., 1993; Chubinishvili et al., 1995).

It is not entirely clear how variation in population size relates to spatial structure (Murdoch, 1994). The goal of the present study was to analyse this problem using a population of the Caucasian brown frog (*Rana macrocnemis*). We tried to resolve the following questions: (1) is the variation in size of the entire population and of individual spatial units within this population density-dependent? (2) If the variation in population size is density-dependent, is it regulated during the larval development or after metamorphosis? (3) Are the dynamics of separate spatial units independent or related? (4) How do the dynamics of separate breeding sites affect the dynamics of the whole population?

MATERIAL AND METHODS

NATURAL HISTORY

Rana macrocnemis belongs to the subgenus Rana (Rana) which has a wide Holarctic distribution (Dubois, 1993; Green & Borkin, 1993). The reproductive biology of R. macrocnemis is similar to that of closely related species (R. temporaria, R. dalmatina, R. sylvatica). Favourable breeding sites are temporary pools. Spawning starts after the melting of snow around breeding sites and is completed in two-three weeks. A female deposits a single egg-clutch containing 200-3500 eggs in a season (Tarkhnishvili, 1993). After the completion of spawning, frogs disperse throughout the terrestrial habitats. They can move at least 5-6 km from a breeding site (Tarkhnishvili, unpublished data). Breeding period, fecundity and terrestrial habits vary in different parts of the species' range (Papanyan, 1961; Kalabekov, 1973; Molov & Ishchenko, 1973; Effendiev & Ishchenko, 1974; Velieva, 1977; Tertishnikov, Logachova & Kutenkov, 1979; Ushakov & Tusnolobova, 1987).

STUDY AREA AND THE DISTRIBUTION OF BREEDING SITES

The study was conducted in the canyon of the river Nedzura (Borjomi district of Georgia), a tributary of the river Mtkvari (Kura). The length of the canyon exceeds 15 km (Fig. 1). Elevation ranges from 900-1000 m (main river bed) to 1200 m (breeding sites situated on mountain slopes). Annual precipitation reaches 1000 mm. The canyon is surrounded by slopes with mixed forest. A few springs feed into the river. A long the river and stream banks, dominant trees are *Alnus barbata, Acer campestre*, and *Carpinus caucasica*; on the slopes – *Abies nordmanniana, Picea orientalis,*

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FIG. 1. Map of the study locality. The River Nedzura and its tributaries are shown. Dots indicate separate pools - breeding sites of *R. macrocnemis*. Positions of sites 2, 10, 44, 38-40, 74-85, 90 and 111-139 indicated with corresponding figures. Broken line indicates the border between upper and lower sub-populations.

Carpinus caucasica and Fagus orientalis. Breeding sites of R. macrocnemis are shared with seven other amphibian species: Mertensiella caucasica, Triturus vittatus ophryticus, Pelodytes caucasicus, Bufo viridis, B. verrucosissimus, Hyla arborea shelkownikowi and Rana ridibunda. A detailed description of the study area is given elsewhere (Tarkhnishvili, 1993, 1994; Gokhelashvili & Tarkhnishvili, 1994).

Breeding sites of frogs were pools and puddles situated along the banks of the river and streams (Fig. 1). The sizes of individual breeding sites ranged from 2-3 to several thousand litres. During the study period, spawn clumps were recorded at 139 breeding sites. Frogs spawned at 26 of these sites in every year of the study. At other sites, frogs spawned occasionally. 64 breeding sites were in the lower part of the canyon, about 5 km along the lower reaches of the Nedzura (hereafter referred to as the 'lower sub-population'). Seventy-five breeding sites were in the upper reaches of the river (the 'upper sub-population').

The individual breeding sites were rather randomly distributed throughout the study area, although some clearings and pastures had several puddles situated close to each another. Especially dense aggregations of breeding sites are shown in Fig. 1.

ANALYSIS OF THE POPULATION STRUCTURE AND DYNAMICS

Over six consecutive years (1990-1995), each breeding site of R. macrocnemis was surveyed every fifth day during the spawning period. Because embryonic development lasted approximately ten days, we were able to count all of the deposited clutches. The total number of clutches was assumed to equal the number of reproductive females. For each individual clutch, the date of deposition and the position of the spawning site were recorded. In 1989, only 13 breeding sites from the upper sub-population were studied.

For 131 clutches collected from all sites in 1989, and for 512 clutches in 1990, the number of eggs per clutch was counted. The methods for the analysis of clutch size and other reproductive characteristics of the study population (including the correlation between female body size, fecundity and egg size) are described elsewhere (Tarkhnishvili, 1993). The total number of eggs deposited in each pond was then estimated as the product of the number of clutches deposited and the average number of eggs per clutch. The number of eggs in each small (less than 600-800 eggs) or large (more than 2500 eggs) clutch was counted individually.

In 1989-1990, the volume of each spawning site was estimated with the half-ellipsoid volume formula: $v = (\pi a b c)/6$, where a and b were maximum length and width of a pool, each divided by 2, and c was its depth. The preferences of frogs for pools of different volumes was estimated with the electivity index of Ivlev (1961): $J=(P_i - P_i^*)/(P_i + P_i^*)$, where P_i^* was the percentage of water bodies of size class i in the environment, and P_i was the percentage of spawn clutches deposited in ponds of this size class. This index was also used to evaluate the level of discrimination between breeding sites of five different types: (1) shallow, well-illuminated pools; (2) pools with a slight current, located in open places; (3) shady, seepage pools in forest, with relatively cold water; (4) cold pools with a slight current, in forest; and (5) brooks.

For the analysis of embryonic and larval mortality (carried out in 1989 and 1990), four sets of data were used: the number of eggs at a breeding site; the number of dead embryos per clutch (for all clutches in ponds containing 1-10 clutches and for ten clutches in ponds containing >10 clutches); the number of tadpoles at a site after the beginning of active feeding (stages 20-25 - Gosner, 1960; generally 3-5 days after hatching was completed); and the number of tadpoles prior to metamorphosis (stages 40-41; generally 2-4 days prior to the beginning of metamorphosis at a site). The latter figure (number of larvae prior to metamorphosis) was used as an index of the number of metamorphs at a site. Moreover, for sites which dried up before completion of metamorphosis, the date of desiccation was recorded. Since metamorphosis was completed in less than one week at most of the breeding sites, individual differences in the developmental rates were not taken into account. The number of active tadpoles was evaluated by three different methods: (1) for the smallest pools (three in 1989 and five in 1990), we simply counted all tadpoles observed at a breeding site; (2) for two shallow pools with the largest water surfaces, the number of tadpoles was counted in each of ten plots of 50 x 50 cm each, the average density per unit pond surface was calculated, and the total number of tadpoles was estimated as a product of the average density and the entire pond surface area; (3) for the other breeding sites (including all pools where metamorphosis was completed) the number of tadpoles was estimated using a mark-recapture technique. We stained tadpoles with neutral red (Guttman & Creasey, 1973), and recaptured them within 4-24 hours of marking (before traces of dye disappeared). A short time interval between marking and

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recapture excluded the effect of ontogenetic behavioural changes which could bias the estimated number of tadpoles. The number of tadpoles per site was evaluated using the inverse Petersen index (Caughley, 1977). Recaptures were made until the number of marked individuals in a sample exceeded the figure that ensures a standard error value of less than 10% (Caughley, 1977).

The relative mortality rate for a stage of development was evaluated as the difference between two consecutive estimates, divided by the initial number and multiplied by 100: $Q_x = (N_o - N_v)/N_o x 100$.

AGE STRUCTURE OF REPRODUCTIVE POPULATION

For some of the adult frogs, we estimated age with skeletochronological standard methods (e.g. Kleinenberg & Smirina, 1969; Castanet & Smirina, 1990). The number of analysed individuals is given in Table 5. Sections (25 μ m) of the second phalanx of the fourth toe were stained in Böhmer haematoxylin. Observations on marked individuals indicated that frogs in the study area reach reproductive size (snout-urostyle length > 50 mm) before the second hibernation. Consequently, the first line of arrested growth (LAG, year ring) had a markedly smaller width than the rest of the LAGs and could be easily distinguished. In general, the first LAG was partly or fully resorbed in adults. The LAG representing the last hibernation was not expressed, since we collected samples in early spring (Gokhelashvili & Tarkhnishvili, 1994). The age of individual frogs was calculated as n+2 (where n is the number of 'full' LAGs) or (rarely) n+1, depending on degree of resorbtion of the first LAG the (Gokhelashvili & Tarkhnishvili, 1994). Age distribution was separately estimated for the two sub-populations and the entire population in 1992, 1993 and 1994. Samples were compared using Kolmogorov-Smirnov lambda-tests and Fisher's angular method (arcsin-transformation of frequencies prior to the pairwise comparison - Sokal & Rohlf, 1995), recommended for comparison of frequencies in samples of small size (Zaitsev, 1984). Multiplying the percentage of separate age cohorts by the total number of clutches in the two sub-populations (see below) and the population as a whole, the total number of reproductive females in a cohort was estimated. The age of first reproduction can vary, but the modal age cohort was assumed to be the greatest age for first reproduction. Mortality rates were estimated as $(N_{i,j} - N_{i+1,j+1})/N_{i,j}$

where N_{ij} was the number of frogs of the age class *i* in year *j* and N_{i+1j+1} was the number of the following age class in the next year (where *i* was greater than or equal to the modal age of the analysed group).

POPULATION REGULATION HYPOTHESES

To detect temporal trends in the dynamics of individual breeding sites, sub-populations and the entire population, the approach of Varley & Gradwell was applied (Southwood, 1996). Prior to the analysis, the number of clutches was *ln*-transformed. The *k*-value, indicating the change in number between two consecutive years, was estimated as: $k = ln N_{i} - ln N_{i}$ For individual breeding sites, the two sub-populations and the whole population, the measure of density dependence, b_i , was estimated as the coefficient of regression of k on $ln N_{.}$ If $0.5 \le b \le 1.5$, the dynamics of a spatial grouping were assumed to be density dependent; if b < 0.5, dynamics were assumed to be density independent; and if b>1.5, the dynamics were assumed to result from an overcompensating effect (Southwood, 1996). To estimate the statistical significance of the values of b obtained, the correlation coefficient between k and ln N, was calculated.

RESULTS

SPATIAL STRUCTURE

The number of breeding sites varied from 73 - 97 between years. The number of sites in the lower sub-population declined from 49 in 1991 to 29 in 1993. The number of sites in the upper sub-population increased from 43 in 1990 to 62 in 1995.

On average, 11.5 ± 0.6 clutches per year were deposited at each site (range: 1-113; CV=123 %; N=560; range of means: 11.32-13.44 for different years; Fig.2). The distribution of clutches throughout breeding sites was strongly aggregated. Some 58% of clutches were deposited at 3.9% of breeding sites, each of which contained 20 or more clutches (Fig. 1). Frogs preferred well-illuminated, warm, shallow pools with a volume of more than 160 l (type 1), and avoided spawning in cold, slowly running waters, and in pools with a volume of less than 20 l (Table 1).

DYNAMICS OF REPRODUCTIVE FEMALES

The numbers of clutches found at each breeding site and at the two sub-populations are shown in Table 2

TABLE 1. Electivity of spawning sites of different type and volume. Data for 1989 and 1990 are pooled. Types of spawning site:
(1) shallow, well-illuminated pools; (2) pools with a slight current, located in open places; (3) shady seepage pools in forest, with
relatively cold water; (4) cold pools with a slight current, in forest; and (5) brooks.

Type of spawning site	1	2	3	4	5
Electivity index	+0.12	+0.06	-0.09	-0.10	-0.71
Volume of spawning site	<20	20-160	160-1280	>1280	
Electivity index	-0.31	+0.05	+0.26	+0.28	



FIG. 2. The distribution of breeding sites according to the number of deposited spawn clutches, using combined data for 1989-1995.

TABLE 2. Dynamics of clutch numbers for the entire population (EP), and lower (LSP) and upper (USP) sub-populations during the study period.

	1990	1991	1992	1993	1994	1995
LSP	479	539	352	264	254	219
USP	546	560	516	798	645	927
EP	1025	1099	868	1062	899	1146

and Fig. 3. The total number of clutches across all sites varied between 868 and 1146. The average number of reproductive females was 1017, with CV=11%. During the course of the study, the number of clutches declined within the lower sub-population, but increased for the upper sub-population.

MORTALITY FROM EGG TO METAMORPHOSIS

Eggs and larvae did not survive in 43 pools in 1989 (78% of breeding sites) and in 79 pools in 1990 (89%) (Fig. 1). From 1991-1995, metamorphosis never took place at more than 20 breeding sites. Desiccation was the commonest cause of mortality. The total number of clutches at sites that desiccated before metamorphosis



FIG. 3. Dynamics of clutch numbers at 139 individual breeding sites. Sites 1-64 belong to the lower sub-population, sites 65-139, to the upper sub-population. Numbers <10 not shown.

was 118 (43.1%) in 1989 and 796 (77%) in 1990. In other breeding sites, the average survival rate from egg to metamorphosis was 5.99% (0.03-23.3%, SE=5.8). The total number of surviving offspring was 4.45% from deposited eggs in 1989 and 3.01% in 1990 (Table 3). The distribution of clutches did not differ between desiccated and successful breeding sites: χ^2 =10.2 (1989), χ^2 =4.0 (1990) (*N*=11, *P*>0.05). The distribution of metamorphs throughout individual sites and plots was even more aggregated than the distribution of eggs. In 1989, 82% of the new generation emerged from two sites; in 1990, 70% metamorphosed from a single site (Table 4).

In 1989, the initial density (number of eggs per unit of water volume) was significantly correlated with mortality rate between hatching and active feeding for 32 breeding sites: r=0.41, P<0.05. For ten sites where metamorphosis was completed, the mortality rate between the beginning of active feeding and metamorphosis was related to the density of newly hatched tadpoles: r=0.68, P<0.05. The regression was curvilinear and the correlation between log-transformed values reached 0.98 (P<0.01). However, the density-dependent mortality was undercompensating: the number of offspring was positively related to the

TABLE 3. Output of the population, in terms of the number of clutches deposited, egg and larval survival, and offspring number. Combined data for 1989 and 1990. Nc, the total number of clutches deposited; Ne, the total number of eggs; Pe, survival of eggs, %; Pl, survival of larvae before active feeding, %; Pt, survival of tadpoles before metamorphosis, %; Pem, survival from egg to metamorphosis, %; Nm, the number of metamorphosing tadpoles. A, combined data for all breeding sites; B, sites which did not desiccate before metamorphosis; C, sources. Whole population (1990), studied part of the population (1989).

	Ne	Ne	De	Pl	Pt	Dem	Nm
	INC	INC	re	11	11	Fem	INIII
1989	274	401,136	61.2	31.0	-	4.45	17,845
1990	1025	1,518,168	?	?	?	3.01	44,600
1989	156	242,619	88.4	14.3	58.3	7.36	17,845
1990	241	315,368	?	?	?	14.14	44,600
1989	74	152,321	87.4	15.6	78.3	10.67	16,247
1990	150	207,467	?	?	?	18.75	38,903
	1989 1990 1989 1990 1989 1990	Nc 1989 274 1990 1025 1989 156 1990 241 1989 74 1990 150	Nc Ne 1989 274 401,136 1990 1025 1,518,168 1989 156 242,619 1990 241 315,368 1989 74 152,321 1990 150 207,467	Nc Ne Pe 1989 274 401,136 61.2 1990 1025 1,518,168 ? 1989 156 242,619 88.4 1990 241 315,368 ? 1989 74 152,321 87.4 1990 150 207,467 ?	Nc Ne Pe Pl 1989 274 401,136 61.2 31.0 1990 1025 1,518,168 ? ? 1989 156 242,619 88.4 14.3 1990 241 315,368 ? ? 1989 74 152,321 87.4 15.6 1990 150 207,467 ? ?	Nc Ne Pe Pl Pt 1989 274 401,136 61.2 31.0 - 1990 1025 1,518,168 ? ? ? 1989 156 242,619 88.4 14.3 58.3 1990 241 315,368 ? ? ? 1989 74 152,321 87.4 15.6 78.3 1990 150 207,467 ? ? ?	Nc Ne Pe Pl Pt Pem 1989 274 401,136 61.2 31.0 - 4.45 1990 1025 1,518,168 ? ? ? 3.01 1989 156 242,619 88.4 14.3 58.3 7.36 1990 241 315,368 ? ? ? 14.14 1989 74 152,321 87.4 15.6 78.3 10.67 1990 150 207,467 ? ? ? 18.75

TABLE 4. Reproductive output of individual breeding sites and sub-populations. Only those sites where some larvae reached metamorphosis are shown. #BS, number of a breeding site (code); Ncl, number of clutches deposited at a site; Nm, number of individuals that reached metamorphosis; Qem, percentage survival from egg to metamorphosis; %C, percentage input of a plot to a new cohort. LSP, lower sub-population; USP, upper sub-population. T, total figures for respective sub-populations.

1989							14							
	LSP									TLSP	USP			
#BS	2	10	14	15	17	18	31	32	44	50	53	59	1-64	74
Ncl	34	20	4	13	3	5	22	4	7	6	4	5	273	7
Nm	8964	4109	291	1174	105	10	687	2	~200	6	364	119	17831	14
Qem	12.7	8.5	4.3	4.3	2.3	0.12	3.1	0.08	19.2	0.14	6.1	2.4	3.7	0.2
%С	50.3	23.0	1.6	6.6	0.5	~0	3.9	~0	11.2	~0	2.0	0.1	~100	~0
1990														
	LSP TLSP			USP						TUS	SP			
#BS	44	45	59	1-64		80	90	91	92	93	113	65-13	39	
Ncl	42	12	26	413	15	10	93	4	7	9	23	61	2	
Nm	~5000	102	1800	6902	3000	500	30903	1000	1000	250	1000	3765	53	
Qem	8.0	0.92	6.22	1.1	14.2	4.4	23.3	17.5	10.0	1.9	3.0	4	.2	
%С	11.2	0.2	4.0	15.5	6.7	1.1	69.4	2.2	2.2	0.5	2.2	84	.5	

number of deposited eggs. The correlation of log-transformed data, combined for 1989 and 1990, reached 0.99; the regression is described by the allometric equation $y=0.00039x^{1.52}$, where y is the number of tadpoles prior to metamorphosis and x is the number of deposited eggs (Fig.4).

SPATIAL DISTRIBUTION OF METAMORPHS AND ADULT FROGS

The number of adult females in each of the two subpopulations was dependent on the number of metamorphs in the same sub-population 2-3 years earlier. In 1989, the majority of surviving offspring came from three pools in the lower sub-population (Table 4).



FIG. 4. The relationship between the number of eggs deposited at a site and the number of tadpoles reaching metamorphosis. Only those breeding sites where metamorphosis was observed are included.

In 1991, the number of clutches in this sub-population increased 1.13 times, whereas those in the upper sub-population increased 1.02 times (in comparison with the previous year). In 1990 the majority of surviving offspring came from a single pond, no. 90 (upper sub-population; Table 3). Between 1990 and 1993, the number of clutches in the upper sub-population increased 1.46 times, whereas those in the lower sub-population decreased by almost a half (Table 2, Fig. 3).

AGE DISTRIBUTION

The age structure of reproductive frogs is shown in Table 5. The age of the vast majority of individuals did not exceed five years. Inter-sexual differences were insignificant (Kolmogorov lambda <1.36, P>0.05 for each year). The age distributions of frogs from the lower and upper sub-populations did not differ significantly, according to Kolmogorov's lambda, but the differences were significant according to Fisher's angular test. In 1993, the proportion of 3-year-old frogs (the cohort originating in 1990) was significantly higher in the upper sub-population: F = 6.20, P < 0.05, df = 52. Mortality rates differed between cohorts and between the two sub-populations. Survival rates in cohorts that originated in 1989 and 1990 were, respectively, 23 and 27% per year during the first three years of life; during the following year, mortality rates reached 35% for the cohort originating in 1990 (upper sub-population) and 43% for the cohort originating in 1989 (lower sub-population).

TEMPORAL TRENDS IN THE DYNAMICS

The factor *b* for the whole population reached 1.93 (P<0.05). The corresponding values were 0.15 for the

TABLE 5. Age distribution of reproductive frogs in 1992-1994. Percentages of specimens in different age classes and the mean ages of reproductive animals are shown. N, sample size; figures for modal age classes are shown in bold type; m, males; f, females; Nf, the absolute numbers of females of different age classes, calculated as the products of the percentages and the total numbers of clutches deposited.

sex	year	N	Age classes (years)							SD
			2	3	4	5	6	7		
(1) Whole population										
m	1992	50	42	42	10	6			2.78	0.85
m	1993	42	26	48	14	10	2		3.13	0.91
m	1994	83	11	29	41	19			3.68	0.90
f	1992	18	22	39	28	11			3.39	0.98
f	1993	12	8	25	42	8	8	8	4.03	1.10
f	1994	14	14	29	36	14	7		3.71	1.14
Nf	1992		191	339	243	95				
Nf	1993		86	262	442	86	86	86		
Nf	1994		126	261	323	126	63			
(2) <i>Lov</i>	ver sub-po	pulatio	n							
m+f	1992	32	53	38	9				2.56	0.66
m+f	1993	34	32	26	29	9	4		3.27	1.12
m+f	1994	31	19	38	29	14			3.00	1.02
Nf	1992		186	134	32					
Nf	1993		83	69	77	24	11			
Nf	1994		48	96	74	36				
(3) Upj	per sub-po	pulatio	n							
m+f	1992	26	37	41	11	11			2.96	0.96
m+f	1993	20		60		20	10	10	4.10	1.45
m+f	1994	66	8	21	48	21	2		3.88	0.90
Nf	1992		190	212	57	57				
Nf	1993			478		160	80	80		
Nf	1994		52	135	310	135	13			

lower sub-population was 0.15, and 0.81 for the upper sub-population (both P < 0.05). Thus, the variation in size of the lower sub-population was density-independent; of the upper sub-population density-dependent, and for the entire locality density-dependent with overcompensation. For individual breeding sites, no significant density-dependence was observed.

DISCUSSION

The study population has a sources-sinks type of spatial organization (Pulliam, 1988, 1996). Every year, a few breeding sites (sources) provided the majority of metamorphs, while at the other breeding sites all or almost all larvae died before metamorphosis. Successfully breeding females represented 15-27% of the population. The spatial position of sources varied from year to year. The dynamics were density-dependent and the range of variation was lower than in many other amphibian populations (Gill, 1978; Ishchenko & Ledentsov, 1987; Berven & Grudzien, 1990; Sinsch &

Seidel, 1995). Could the sources-sinks type of spatial structure be responsible for the observed stability? The key to this question is in the analysis of the dynamics of individual spatial units.

Density-dependent population dynamics could be connected with either density-dependent mortality during the larval period (Shoop, 1974; Tarkhnishvili & Pyastolova, 1985; Severtsov & Surova, 1989; see Wilbur, 1996 for review), or with density-dependent mortality, immigration and individual growth rates in the terrestrial stages (Ishchenko, 1983, 1989; Lyapkov, 1995). Mortality of larvae was density-dependent at sites which did not desiccate. However, most of the breeding sites desiccated. This reduced the importance of density-dependence for mortality, as in many other amphibian populations (Semlitsch, 1987; Cooke, 1975; Kuzmin & Godina, 1986; Albers & Prouty, 1987). Moreover, a strong positive correlation between the numbers of eggs and metamorphs was recorded: this indicated that the variation in larval mortality between breeding sites was not regulating numbers. Consequently, small variations in the population size appear to be connected with demographic events that occured after metamorphosis.

A plausible explanation is that dispersal prevented both over-population of sources and extinction of sinks. The number of offspring at an individual site influences the number of reproductive females in all neighbouring sites a few years later. For instance, the total number of frogs in the upper sub-population increased significantly (by 46%) three years after a year when a large number of juveniles emerged from breeding site no. 90. This population growth does not apply to site no. 90 itself, i.e. most of the offspring from 1990 did not return for breeding to their pond of origin. A similar situation was observed for the lower sub-population, whose size increased by 12% after two years, since the most productive breeding sites were nos. 2, 10 and 44. Analysis of the age structure of reproductive frogs supports a relationship between the number of offspring that developed in sources and the number of adults that appeared in the same sub-population over following years.

Sites 111-139, located within a large, well-illuminated pasture (Fig. 1), were the most important recipients of the cohort which originated from site no. 90 and a few adjacent pools. In 1993, the number of reproductive females at these sites increased by 237 in comparison with the previous year. The number of females in another, adjacent group of sites (74-85, which were located in forest) increased by just 62 (Fig. 3). Collectively, these breeding sites ensured the survival of 3.3% of the deposited eggs, a much higher rate than sites 111-139. Was the negative association between survival of larvae and immigration rates accidental? Sites 111-139 are shallow, warm, well-illuminated pools, which frogs prefer for spawning. Most of these pools desiccated soon after spawning. Thus, preferences of reproductive females did not ensure an increase in reproductive fitness. Other observations supported the absence of association between preferred and effective sites. For example, at site no. 2 spawning took place until 1995, though no larvae survived there after 1989. The number of clutches at sites 38-40 remained high between 1992 and 1995, though no successful metamorphosis was observed there until 1995. All these sites are shallow and warm pools. It is hard to explain the preference for non-optimal sites. Possibly these sites provide 'comfortable' conditions for breeding adults that simply prefer to stay in relatively warm water (or perhaps survive better if water is not so cold), and this factor has a stronger impact than the survival of eggs and larvae on the selection of breeding sites.

Ray & Hastings (1996) showed that, for insects, density-dependence in metapopulations is more often detected for mobile than for immobile stages of the life cycle. Perhaps this conclusion also applies to amphibians. Simulation models support the point that local migrations can increase the density dependence of a metapopulation (Hanski, Foley & Hassell, 1996; Howe *et al.*, 1991). This process is accelerated if sinks attract reproductive specimens more than sources (such types of sinks Pulliam (1996) called 'traps'). In the study area, presence of traps determines the main directions of dispersal (mainly from sources to sinks) and strengthens density-dependence in overall population dynamics.

An additional mechanism of regulation appears to be density-dependent individual growth and mortality. In 1990, the source was in the upper sub-population and twice as many metamorphs emerged than in 1989, when the source was in the lower sub-population. Consequently, frogs that metamorphosed in 1989 from the lower sub-population began to reproduce (on average) after two hibernations, whereas frogs that metamorphosed in 1990 began to reproduce after three hibernations (Table 5). A similar postponement of maturation in cohorts with a high initial density was recorded for Rana arvalis (Ishchenko, 1989; Ishchenko & Ledentsov, 1987). Late-maturating cohorts had lower annual mortality rates than early-maturating cohorts, as in other brown frogs (Berven, 1988; Ishchenko & Ledentsov, 1987).

It is notable that the number of frogs was more stable and well-regulated for the entire population than for the two separate sub-populations. The decline of the lower sub-population was caused by anthropogenic influences in the 1990s, when some breeding sites were destroyed. The reasons for a simultaneous increase in the upper sub-population remain unclear. The regulatory mechanisms described above do not provide a plausible explanation because the dispersal rates between lower and upper sub-populations appear to be low. Because the number and sizes of breeding sites in the upper sub-population increased in the course of the study, this phenomenon might be explained by an examination of hydrological data for the area.

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BOOK REVIEWS

Contributions to the Herpetology of the Belgian Congo. Karl P. Schmidt and G. K. Noble. (1998). 780 pp., 141 photos, 1 colour plate. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA (in cooperation with the Herpetological Association of Africa). Facsimile Reprints in Herpetology. US\$65.00 (cloth).

The Gigantic Land Tortoises of the Galapagos Archipelago. John Van Denburgh. (1998). 290 pp., 205 photos. Society for the Study of Amphibians and Reptiles, Ithaca, New York, USA. Facsimile Reprints in Herpetology. US\$55.00 (cloth).

The two latest additions to the SSAR reprints series are largely taxonomic works from expeditions in the first quarter of this century. The Contributions contains a collection of three long papers from the Bulletin of the American Museum of Natural History, 1919-24; two on reptiles by Karl Schmidt and one on amphibians by G. Kingsley Noble. These form the herpetological results of the AMNH Congo expedition of 1909-1915. The Giant Land Tortoises was originally published in the Proceedings of the California Academy of Sciences in 1914, and is based on the CAS Galapagos expedition of 1905-6. The two works have many similarities, not least that none of the authors participated in the expeditions or saw the animals in the field. However, the interest of the two books, and their present utility are completely different.

The AMNH Congo expedition was a massive enterprise lasting six years. It covered about 15 000 miles on foot (the only alternative at the time was water transport in native canoes, which would have damaged specimens), involving a total of over 38 000 porters, and shipping 54 tons of material to the USA, including over 4000 specimens of reptiles and amphibians. The expedition is described in a brief paper by H. F. Osborn (1919), including a detailed map, which is not included in the SSAR volume. Much of the charm of the book derives from the field notes and photographs of Herbert Lang, the expedition leader; unusually for a taxonomic work of the period, many of the photographs are of living animals. The colour plate shows pattern variation in chameleons, which must have been novel at the time, though now familiar to all.

Much of the text is purely taxonomic, with details of size, scalation, and colour patterns, though there are also descriptions of gut contents and reproductive status, and distribution maps (often Africa-wide) of many of the reptile species. There are also biogeographical essays by both Schmidt and Noble, which were among the first such analyses of the African herpetofauna. Non-taxonomists will be most interested in the field notes and photographs, which are particularly good on the lizards; most snakes were photographed dead, and there are only sparse field notes on amphibians. There is a striking picture of the Congo green lizard Gastropholis echinata, a large slender lacertid with a unique ruff of long spiny scales on the tail. The function of these scales is still unknown; they may enhance climbing ability (Arnold, 1989), although Lang's field notes indicate that they are sexually dimorphic, and so possibly involved in courtship. There are several descriptions of defence behaviour, including the 'jackknife' saltatory posture of the limbless lizard Feylinia currori, parotid venom squirting (to a distance of several feet) in Bufo superciliaris, and a description of a typical stabbing bite by Atractaspis (Lang was lucky not to lose a finger tip). Many field notes include interesting capture details, such as Varanus exanthematicus extracted from a hole in an old termite mound after biting the end of a spear, and Mabuya quinquetaeniata tangled in traps of tiny grass stalks set by children.

I was particularly interested in the tortoises. Kinixys belliana was thought to be nocturnal ("like most land turtles") because they were so rarely seen during the day. In fact, it is the lack of nocturnal activity in tortoises which is surprising, especially those limited by high temperatures such as the desert tortoise or the Aldabran giant tortoise, although nesting may be prolonged after dusk. K. belliana aestivated in the dry season in savanna, while K. erosa was active all year round in the rain forest. Aestivating tortoises were found with hunting dogs, dug up with spears and cooked - they were preferred to chicken. Lang provides anthropological notes on many species; Agama agama was also eaten, and the head kept as an aphrodisiac amulet or burnt and powdered in banana wine. Bones and shell parts of K. belliana were worn extensively, and the story of a race between the turtle and the elephant ("with the generally accepted ending") was widely known, presumably equivalent to the tortoise and the hare. This fable is appropriate to Kinixys species, which move relatively long distances for small tortoises; K. spekii walks about 400 m per day in cool weather, further than the average American (350 yards: Bryson, 1998).

The value of the SSAR reprint is enhanced by the introductions by Donald Broadley (reptiles) and John Poynton (amphibians). Both give tables of current nomenclature, references to the Congo herpetofauna since 1963 (when the last bibliography was published), and a list of species currently thought to occur there, tabulated into five regions. Only a fraction of the species which are now thought to be present were collected by the AMNH expedition (46% of the 290 reptiles and 22% of the 232 amphibians), partly because they did not sample two of the five Congo zoogeographic regions. Nevertheless, both Broadley and Poynton endorse the current value of the *Contributions* to tax-onomists of the region.

The CAS expedition to the Galapagos collected 266 tortioises, and John Van Denburgh's book includes descriptions and photographs (all of dead animals) of the forms from the different islands. The book begins with a brief history of the early exploitation of the giant tortoises. As with Kinixys, they were highly recommended: "After once tasting the Galapagos tortoise, every other animal food fell greatly in our estimation". The introduction by Peter Pritchard draws attention to the modern nature of Van Denburgh's analysis, with tables of morphometric data and graphs, although these do not make for easy reading. Pritchard gives the current nomenclature of the Galapagos tortoises, though their taxonomy remains controversial. The book also gives brief field notes on each population, with some interesting observations of diet and behaviour made by the expedition's herpetologist J. Slevin. Most of the field notes are, however, limited to the collecting and skinning of the tortoises. These descriptions may be unpalatable to modern readers, although Pritchard points out that the CAS expedition did not fatally reduce the numbers of any Galapagos population.

The production quality of both of these reprints is high, and the SSAR is to be congratulated on making them available again. The *Contributions* is the more valuable work, and will be of interest to all those working on the African herpetofauna. The *Gigantic Land Tortoises* will be of interest to every herpetologist who visits the Galapagos. The SSAR Facsimile Reprints in Herpetology series, which now extends to about 50 titles, is thus still finding useful works to reprint. Most of the titles so far have been taxonomic; reprints of some of the classics of functional herpetology would be welcome, such as Benedict's *Physiology of large reptiles* (1932) or the herpetological parts of Walls' *Vertebrate eye* (1942).

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Adrian Hailey British Herpetological Society Herpetological Bibliography of Indonesia. Indraneil Das. (1998). 92 pp. Krieger Publishing Company, Malabar, Florida. £20.50 (cloth).

Bibliographies are out of date the moment they are published; this is the inevitable fate of the printed word and further proof, if it were ever needed, of the advantages of the computer database. However, this is not a facility available to everyone and there is debate over the permanence, and completeness, of Internet-sourced databases. I think it is a safe bet that many readers will first reach for a book when initiating research or simply checking on a reference.

The title is somewhat misleading in that data for regions outwith the turbulent political boundaries of Indonesia - a sprawling chain of islands that spans two million square kilometres - are included, and yet, New Guinea, the World's second largest island and half of which is Indonesian (Irian Jaya) is excluded. Whilst no rationale is offered in this volume for this omission, South-East Asia is at the cross-roads of the biogeographic regions of Indo-Malaysia and Australasia and New Guinea falls into the latter zone. The author has written extensively on the reptiles and amphibians of this region, for example the "Biogeography of the reptiles of South Asia."

The region covered by this book is described in the introduction as "the archipelago of Indo-Malaysia, stretching from the Andaman and Nicobar Islands eastward to Timor." It is the largest archipelago in the world with 14 000 islands and encompasses territory controlled by India (the Andaman and Nicobar islands), Myanmar (Cocos Islands), Malaysia (Sarawak and Sabah) and the Philippines (Palawan).

The bias of the book is towards natural history and faunistics, and consequently the huge volume of literature on captive husbandry is not included. Similarly the author has chosen not to include the bulk of snake venom research. However, the author has diligently amassed references from every conceivable source spanning the range from popular magazine and scientific journal, to society publications that often have a limited distribution. The latter are rarely included in databases and consequently this book will ensure this wealth of literature can still be exploited. Also included are works that span whole regions such as faunistic lists of the "Orient." The cut-off date is October 1997.

The eyes of the world are focused on Indonesia, not perhaps for the extraordinary richness and beauty of its wildlife, but for the harsh repression of any dissent. In its simplicity this is an impressive, academic contribution that may go some way to revealing another facet of that country and the value of such a work should not be overlooked; it will prove an invaluable aid to anyone working within this region.

David Blatchford British Herpetological Society

INSTRUCTIONS TO AUTHORS

(revised January 1999)

- The Herpetological Journal publishes a range of features concerned with reptile and amphibian biology. These include: Full Papers (no length limit); Reviews and Mini-reviews (generally solicited by a member of the editorial board); Short Notes; controversies, under Forum (details available from the Editor); and Book Reviews. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the Herpetological Journal is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.
- 2. Three copies of all submissions, and illustrations, should be sent to the Scientific Editor. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigour, and the degree of general interest in the subject matter. The Editor's decision will be final.
- 3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. The journal is typeset direct from the author's computer diskette, so all manuscripts should be prepared using a wordprocessor (preferably on a PC-compatible microcomputer). It is not necessary to submit a computer diskette with the initial manuscript, but this will be required in the event of the manuscript being accepted for publication.
- 4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. A sans serif font (e.g. Universe or Helvetica) is preferred.
- 5. The usual rules of zoological nomenclature apply.
- 6. Tables are numbered in arabic numerals, e.g. TABLE l; they should be typed double spaced on separate sheets with

a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

- 7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.
- 8. References in the text should be given as in the following examples: "Smith (1964) stated—"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal.

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

- Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* 2, 206–210.
- Dunson, W. A. (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.
- 9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer diskette ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.
- Proofs should be returned to the Managing Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.
- 11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.
- 12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

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