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CONTENTS

MEEK, R. Thermoregulatory behaviour in a population of Hermann's tortoise (*Testudo hermanni*) in southern Yugoslavia 387

HAILEY, A. Thermoregulation and activity metabolism of the armoured anguid *Ophisaurus apodus* 391

BEEBEE, T. J. C. Possible origins of Irish natterjack toads (*Bufo calamita*) 398

POND, C. M. and MATTACKS, C.A. Anatomical organization of adipose tissue in Chelonians 402

LONG, D. R. Inter-specific comparisons of growth relationships in Chelonians 405

MORRISON, D. A. and GALLAGHER, K. J. Possible use of water turnover rates to estimate food intake in lizards 407

HARRISON, J. D., GITTINS, S. P. and SLATER, F. M. Morphometric observations of smooth and palmate newts in mid-Wales 410

VERRELL, P. A. The responses of inseminated female smooth newts, *Triturus vulgaris*, to further exposure to males 414

ROBERTS, J. M. and VERRELL, P. A. Physical abnormalities of the limbs of smooth newts *Triturus vulgaris* (Short note) 416

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THERMOREGULATORY BEHAVIOUR IN A POPULATION OF HERMANN'S TORTOISE (*TESTUDO HERMANNI*) IN SOUTHERN YUGOSLAVIA

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SUMMARY

Observations have been made on the spring thermoregulatory behaviour of a population of Hermann's tortoise *Testudo hermanni* in southern Yugoslavia. To regulate body temperature *T. hermanni* shuttles between sunlit and shaded areas, but avoids moving extensive distances onto open areas, confining daily movements to the clearing perimeters. Basking takes place throughout the daily period, but is most frequent during the morning. Most tortoises retreat to shaded areas by late afternoon. Locomotory activity, feeding and mating were observed throughout most of the daily period, either in sunlit or shaded areas. Size (carapace length), air temperatures and substrate temperatures do not significantly influence body temperatures. The body temperatures of males, females and juveniles were not significantly different. Although some differences were observed between body temperatures during different periods of the day, the differences were not significant. The maximum body temperature recorded was 34.2°C, this agrees well with the earlier data collected on this species by Meek & Inskeep (1981) and the laboratory study of Cherchi (1956).

INTRODUCTION

Studies on the behaviour of reptilian heliotherms have shown that, in general, diurnal movements are functions of behavioural thermoregulation (Cloudsley-Thompson, 1971, Avery, 1982). However, these animals must also devote time to such non-thermoregulatory behaviour as feeding and mating, while avoiding the critically high temperatures that may lead to their death. Such problems are especially relevant for the terrestrial chelonians, particularly in areas of high temperatures and limited shade where, because they are able to move only very slowly (Jayes & Alexander, 1980) may experience body temperatures very close to their critical maxima (Swingland & Frazier, 1979). This paper is concerned with the daily behaviour and thermal relations of a population of Hermann's tortoise (*Testudo hermanni*) in its scrubland habitat in southern Yugoslavia.

Thermoregulation and thermoregulatory behaviour in Mediterranean chelonians have been investigated in several species. The semi-aquatic forms *Emys orbicularis* and *Mauremys caspica* have been studied by Cherchi (1956) and Meek (1983). Of the terrestrial species, *Testudo graeca* has been the subject of several studies in both laboratory and in the field (Bannikov, 1951; Hutchison, Vinegar & Kosh, 1966; Cloudsley-Thompson, 1974; Lambert, 1981; Meek & Jayes, 1982). Rather less attention has been paid to the other major Mediterranean species *Testudo hermanni*, particularly in wild populations. Cherchi (1956, 1960), Cherchi, Holzer, Scortecci & Serrato (1958) and Thines (1968) investigated the temperature relations of this species in the laboratory, whilst Meek & Inskeep (1981) gathered a random sample of body temperatures from a population of wild *T. hermanni* in southern Yugoslavia. Further observations have now been made on this population and the work has been expanded to investigate daily behavioural patterns.

METHODS

The field work was carried out in early May 1983, in an area of scrubland in southern Yugoslavia (Meek & Inskeep, 1981). Behavioural patterns were monitored by making routine patrols of the study area and recording each located tortoise's behaviour and when possible its body temperature. Since the daily observations of body temperature and behaviour were made over a large area (approximately 1.5 km^2) it is unlikely that a significant number of individual tortoises were sampled twice in one day. Therefore, alterations in behaviour through disturbing the animals, which did take place on a small number of occasions in animals that were basking or inactive, is believed to be negligible. The definitions of behavioural patterns are essentially those given in Meek & Jayes (1982) but with slight modifications. These were: *Basking*, inactive and fully exposed to the sun's rays. *Shade*, located inactive in an area where no sunlight penetrated. *Feeding*, may have taken place in a sunlit or shaded area therefore an additional data point was awarded to either shade or active data to allow for any possible effect on the animal's body temperature. *Active*, animals observed walking around their habitat. This category also includes tortoises observed in courtship or mating behaviour.

Body temperatures (T_b) were recorded from tortoises with carapace lengths ranging from 68–170 mm. Temperatures were recorded with a Whitley electronic thermometer (see Meek, 1983) inserted into the cloaca. Simultaneously air (shaded) and substrate temperatures were recorded; air temperatures 15 cm above the ground, substrate temperatures by inserting the probe 8 mm into the soil. Body temperatures were recorded throughout the day between 09.30 and 18.35 hr mostly under clear skies, although on one morning (6 May) there was some cloud.

RESULTS

BEHAVIOUR

Within its scrubland habitat *T. hermanni* generally avoids moving distances of more than 2–3 m from shade. From 117 observations only one tortoise (0.8%) was located at a substantial distance (12 m) from deep shade. This animal was located at 12.00 hr with a body temperature of 33.9 °C (mass = 622 g) and appeared to be moving off the clearing when located. However, tortoises utilize the clearing perimeters for basking, feeding, locomotory activity and mating. Shuttling between basking and shaded areas was an important part of daily behaviour and formed 63.5% of total observations (basking = 45.2%, shade 18.3%). Active tortoises were located between 10.35 and 16.05 hr either on clearing perimeters (53.8%) or in shade (46.2%). Locomotory activity represented 27.4% of observations, 8.3% of which was mating. Mating took place between 11.15 and 14.45 hr mostly in the shade (75%). Between 10.45 and 16.00 hr tortoises were observed feeding, largely on the clearing perimeters (72.7%).

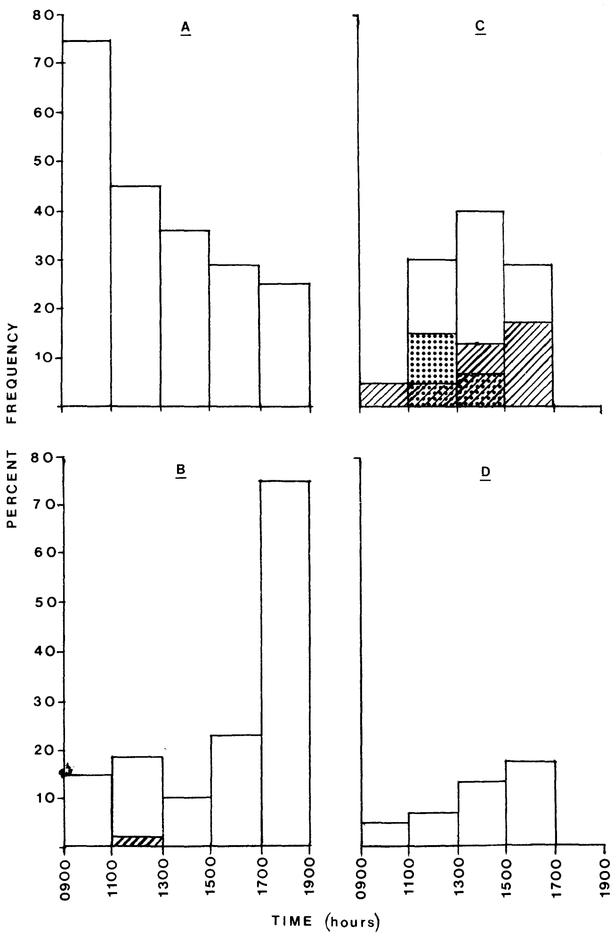

FIG. 1. A series of histograms designed to show the changes in behavioural routines of *T. hermanni* throughout the daily period. These results are based on 131 observations and have been grouped into two-hourly unit periods to show how much time, as a percent of each time period, is spend basking (A), in the shade (B), active (C), and feeding (D), as the day progresses. The stippled areas on active data show the proportion of data which accounts for tortoises involved in courtship or mating. The hatched areas on the histograms B or C show when tortoise were feeding in the shade (B), or in the open (C).

Figure 1 shows these daily behaviour patterns and their relative importance as they occur throughout the daily period when the data are grouped into 2-hourly unit time periods. The histograms show that tortoises bask at all periods of the day, but particularly between 09.00 and 11.00 hr when it represents 75% of the period. As the day progresses however, the amount of time spent basking declines as the animals shift to other types of behaviour. Although tortoises were observed active at most times of the day, their main period for locomotory activity was between 11.00 and 16.00 hr with the peak period between 13.00 and 15.00 hr. There was no clearly defined feeding period, although rather more animals were observed feeding between 15.00 and 17.00 hr than at other time periods.

Figure 2 shows the daily behaviour and thermal history of a small (342 g) male tortoise monitored on 3 May which may be regarded as a typical daily routine at this time of year. This tortoise was first located basking at 09.45 hr with a body temperature of 24.1 °C. Basking was observed until 11.45 hr by which time it had elevated its body temperature to 31.0°C. At 12.15 hr the tortoise was located active beneath dense vegetation (T_b = 32.1°C) and from 12.35–12.50 hr courted a 988 g female in deep shade. Whilst involved in courtship behaviour its body temperature dropped

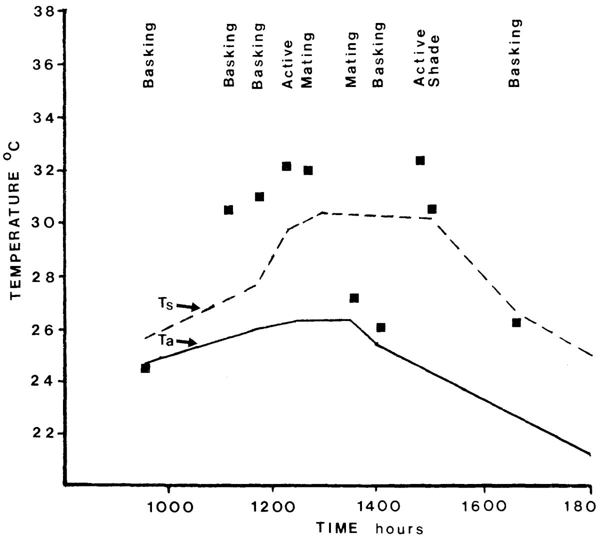

FIG. 2. Daily behaviour and thermal history of a 342 g male *T. hermanni*. The square symbols represent the tortoise's body temperatures. Directly above each data point the corresponding behaviour of the tortoise at the time its body temperature was measured is shown. The broken line indicates substrate temperature (T_s) the continuous line air temperature (T_a). Throughout the daily period this tortoise's body temperature ranged from 24.1°C to 32.8°C ($\bar{x}$ = 29.1°C, SD = 3.09, n = 10).

from 32°C to 27°C. Basking was resumed at 13.05 hr at a body temperature of 26°C and at 14.45 hr the animal was again active in the shade at its highest recorded body temperature of 32.8°C. By 15.00 hr the tortoise was inactive in the shade with a body temperature of 30.3°C. The last observation was at 16.30 hr when it was again basking with a body temperature of 26.1°C. During the daily period the only behaviour not recorded was feeding.

BODY TEMPERATURES

Body temperature data for *T. hermanni* are summarized in Table I. A maximum voluntary body temperature of 34.2°C was recorded from males ($n = 76$), 33.1°C from females ($n = 27$) and 32°C from juveniles ($n = 7$). Comparison of body temperatures of males, females and juveniles by analysis of variance showed no significant difference $F_{2,107} = 1.39$, $P > 0.05$. There was no significant correlation of body temperature with carapace length (Fig. 3), $r = 0.18$, 109 df, $P > 0.05$.

TABLE I. Summary of *Testudo hermanni* body temperatures (°C) in southern Yugoslavia

		Min	Max	$\bar{x}$	95% Confidence limits	n
All data	pooled	20.0	34.2	29.3	6.0	110
	males	21.3	34.2	29.8	5.9	76
	females	20.0	33.1	28.7	6.7	27
	juveniles	27.1	32.1	30.7	3.3	7
Active	pooled	22.6	33.9	29.4	6.2	12
	males	26.7	33.9	30.1	5.9	7
	females	22.6	30.2	27.6	6.7	5
Feeding	pooled	27.5	32.8	30.2	3.5	11
	males	27.5	32.8	30.0	3.6	9
	females	29.6	32.0	30.8	3.3	2
Mating	pooled	25.5	32.0	29.2	4.3	13
	males	27.9	32.0	29.8	3.0	8
	females	25.5	32.0	28.3	5.9	5
Basking	pooled	20.0	34.2	29.5	5.5	55
	males	21.4	34.2	28.9	6.3	35
	females	25.2	33.1	30.0	4.2	15
	juveniles	30.6	31.6	31.2	0.9	5

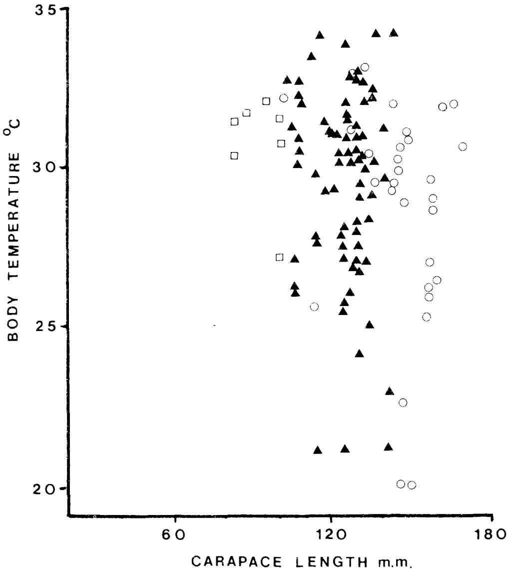

FIG. 3. A graph of body temperatures plotted against carapace length ($n = 110$). The graph shows that tortoise size has no significant effect on body temperature ($P > 0.10$). The symbols represent males (▲), females (○) and juveniles (□).

RELATIONSHIP WITH ENVIRONMENTAL TEMPERATURES

Body temperatures in relation to air and substrate temperatures are shown in Fig. 4. No significant correlation between body temperatures and air temperatures was found, females $r = 0.37$, $P > 0.05$; males $r = 0.14$, $P > 0.10$; juveniles $r = 0.17$, $P > 0.10$. The majority of tortoises (89.1%) had body temperatures higher than air temperature (Fig. 4a). Body tem-

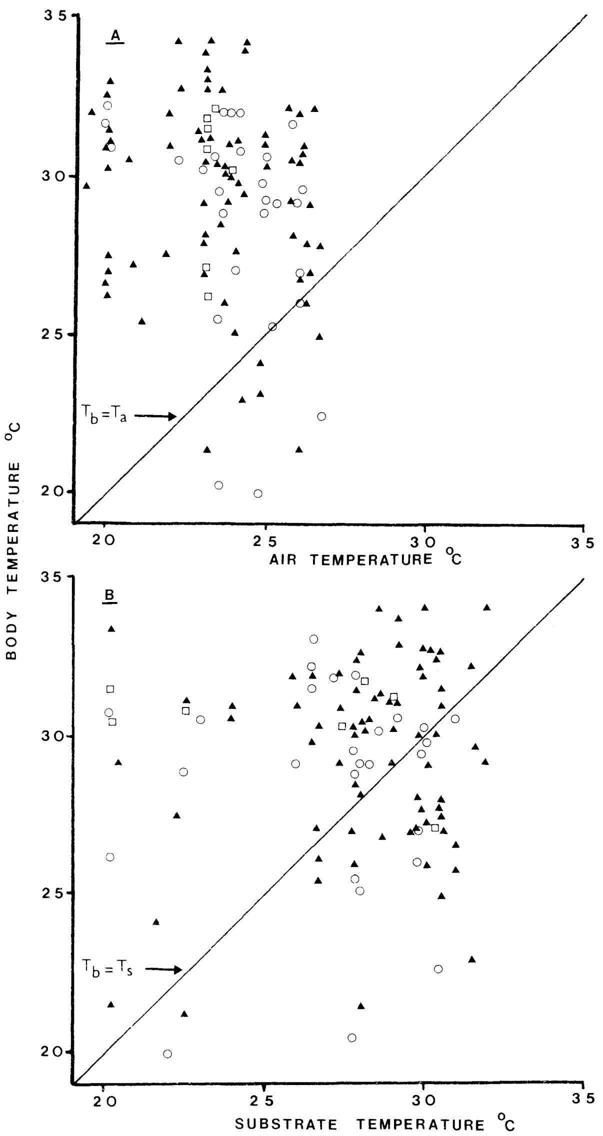

FIG. 4. Graphs of body temperatures ($n = 110$) plotted against (A) air temperatures and (B) substrate temperatures. The lines taken through the data indicate where body temperatures are equal to air or substrate temperatures. The symbols represent males (▲) females (○) and juveniles (□).

peratures were significantly higher than air temperatures, males $t = 13.2$, 75 df, $P < 0.001$; females $t = 4.9$, 26 df, $P < 0.001$; juveniles $t = 10.5$, 6 df, $P < 0.001$.

Figure 4b shows body temperatures in relation to substrate temperatures. The majority of tortoises (68%) were found to have body temperatures higher than substrate temperatures. Males and juveniles were significantly higher, males $t = 2.78$, 75 df, $P < 0.001$; juveniles $t = 2.88$, 6 df, $P < 0.001$, although not females $t = 1.65$, 26 df, $P > 0.10$. There was no significant correlation of body temperatures with substrate temperatures, males $r = 0.16$, $P > 0.1$; females $r = 0.15$, $P > 0.10$; juveniles $r = 0.52$, $P > 0.10$.

BODY TEMPERATURES OF ACTIVE TORTOISES

Table I gives details of the body temperatures recorded from active (walking), feeding and tortoises which were observed mating. A comparison of these data by analysis of variance showed no significant variation between the data sets, $F_{2,33} = 0.28$, $P > 0.05$.

BODY TEMPERATURES IN RELATION TO TIME OF DAY.

Body temperatures were found to be lower and have greater variation during the early morning period from 09.00–11.00 hr ($\bar{x} = 27.5$ °C, SD = 4.0 °C). After this time they increased and showed less variation (11.00–13.00 hr, $\bar{x} = 29.4$ °C, SD = 2.8 °C; 13.00–15.00 hr, $\bar{x} = 29.7$, SD = 3.0 °C). Body temperatures were highest and had the least variation between 15.00 and 17.00 hr ($\bar{x} = 30.3$, SD = 1.9 °C). A comparison of these data, by analysis of variance, however, showed no significant difference in the variation between the time periods when tested at the 95% confidence interval, $F_{3,106} = 2.30$, $P > 0.05$.

DISCUSSION

The monitoring of spring thermoregulatory behaviour of *T. hermanni* in a thermally-diverse habitat has revealed some of the classical patterns of reptilian heliothermy such as pronounced early morning basking, shuttling between sunny and shaded microenvironments and, after body temperatures are sufficiently elevated, feeding, mating and locomotory activity. The results indicate that *T. hermanni* effectively thermoregulates to body temperatures which are largely independent of its thermal environment and by limiting its movements onto extensive clearings, avoids exposure to ecologically lethal temperatures.

The availability of shaded areas has been shown to be of critical importance in the habitats of many species of terrestrial chelonians. Swingland & Frazier (1979) found that the Aldabran giant tortoise *Geochelone gigantea* must seek shade at mid-day on sunny days to avoid internal body temperatures reaching critical maxima of 36–38 °C. They believed that heat-death as a result of over-exposure was the major cause of death of tortoises on their study area. Lambert (1981) and Meek & Jayes (1982) observed that feeding and locomotory activity of *Testudo graeca* were undertaken in shaded areas. In these microenvironments body temperatures may drop as a result of contact with the cooler substratum; excess heat being dissipated through the plastron by blood shunt mechanisms (Mackay, 1964; Lambert, 1981). Andreev (1948, reference in Cloudsley-Thompson, 1971) recorded that *Testudo horsfieldi* retreats to shade when air temperatures reach 34–35 °C; temperatures above 40 °C induce *T. graeca* to move into shaded areas (Lambert, 1981). When temperatures approach lethal limits the desert tortoise *Gopherus agassizi* retreats into its burrow where temperatures are cooler (Woodbury & Hardy, 1948; McGinnis & Voigt, 1971).

Behavioural thermoregulation enables *T. hermanni* to maintain a narrow zone of temperatures for locomotory activity, feeding and mating. These activities take place over a narrower range of temperatures than those recorded from basking tortoises. Tortoise basking at high temperatures could, in part, be a function of post-feeding behaviour. Shuttling between sunlit and shaded areas maintain almost constant body temperatures in the Galapagos giant tortoise, *Geochelone gigantea* (Mackay, 1964; Rodhouse *et al.*, 1979) and for at least part of the daily period in *Testudo graeca* (Lambert, 1981; Meek & Jayes, 1982). Body temperature stability in tortoises is, as has been found in other reptiles, influenced by size (Cloudsley-Thompson, 1971). In large tortoises heat exchange because of lower surface area to volume ratios takes place more slowly than in small tortoises. The body temperatures of a 342 g *T. hermanni* (Fig. 2) fluctuated rather more than those of four larger (640–1770 g) *Testudo graeca* in North Africa (Meek & Jayes, 1982). The relationship between surface to volume ratios and heating rates could influence the statistical results of the relationship between body temperature and size, since there is no direct evidence in this study to show that when the measurements were made, the smaller tortoises had not already achieved maximum body temperatures whilst the larger animals were still heating up. This is to say that if the final daily temperature for each tortoise was plotted against its carapace length it might be possible to produce a significant correlation.

The maximum body temperature of 34.2 °C recorded from *T. hermanni* is in good agreement with the maximum of 34 °C previously recorded by Meek & Inskeep (1981) and with the maximum found in the laboratory studies of Cherchi (1956; 1960). The highest voluntary body temperatures found in both field and laboratory studies of *T. hermanni* do not therefore approach the critical maximum of 39–42 °C or lethal maximum of 44–45 °C recorded for this species (Cherchi, 1956). The mean body temperature of 29.3 °C found in this study and mean of 30.4 °C calculated by Meek & Inskeep (1981) do not differ significantly ($t = 1.25$, $P > 0.10$) and agree well with the general mean recorded for chelonians of 28.4 °C (Brattstrom, 1965).

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THERMOREGULATION AND ACTIVITY METABOLISM OF THE ARMOURED ANGUID *OPHISAURUS APODUS*

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(*Received* 27 *October* 1983)

SUMMARY

The mean body temperature (T_b) of active male *Ophisaurus apodus* in a thermal gradient was 27.7°C (range 24–35°C). Standard metabolic rate (SMR) between 11 and 35°C had an overall Q_{10} of 3.0 (95 confidence limits 2.7 to 3.3) and was 2/3 than expected of a 330 g lizard. The SMR of *O. apodus* was lower and more temperature sensitive than that of English *Anguis fragilis*, after compensation for the size difference. This would be advantageous if high latitude is correlated

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with a lower mean and a greater range of activity T_bs. Such a pattern was found in published records of lacertid T_bs, but not of anguid T_bs. Anguid T_bs are significantly lower and more variable than those of lacertids. Metabolic rate at 11 °C was not altered during hibernation.

O. apodus did not ventilate during induced maximal activity, which was intense burst of ~15 s, and up to 3 min of less intense twisting. Maximal oxygen exchange capacity was ~0.4 ml g^{-1} h^{-1} at 35 °C, similar to that of large skinks. This reflects the cruising forager lifestyle. Anaerobic capacity was 0.55 mg lactate g^{-1} at 25 °C, lower than that of other lizards. This reflects the reliance of *O. apodus* on mineral defence, its osteoderms accounting for 9.1% of live weight; *O. apodus* is 70% as armoured as a similarly sized *Testudo*. The genus *Ophisaurus* includes a range of lifestyles, defensive methods and exercise physiology.

INTRODUCTION

Ophisaurus apodus, the European glass "snake", is the largest legless lizard and one of Europe's largest lizards. Its biology is poorly known; Obst (1981) summarizes the available information. This study aims to extend the taxonomic range of reptiles for which data on activity metabolism are available (Bennett, 1982), and to stimulate further study of this interesting animal.

METHODS

ANIMALS

Seven adult males, mean 330 (250–400) g and 99 (87–106) cm total length, were obtained from Alyki, Greece (40° 22′ N, 22° 38′ E) in the summers of 1980 (three animals) and 1982. They were kept in two well-ventilated 120 × 40 cm vivaria, with heat provided by three 100 W reflector lamps at one end of each cage, on for 9 hr of a 10–11 hr photoperiod. The floor was covered with 6 cm of sawdust, in which the lizards burrowed. Water was always available. Locusts and suckling mice were eaten readily, and the lizards grew slowly. They were allowed to hibernate in constant darkness at 8–12 °C for 7 weeks in the winter of 1982–83, and experiments were performed the following year.

THERMOREGULATION

Two of the larger lizards were forced to swallow a Mini Mitter model V radio pill, size 14 mm diameter × 18 mm and 3.5 g when coated. After a week, body temperature (T_b) was recorded at half hourly intervals on several days, together with the temperature at mid-depth in the sawdust (T_s) at both ends of the cage. The transmitters remained in the gut, 10 cm from the cloaca, for two months, but did not prevent the passage of food. The two lizards were killed and their osteoderm coats removed. Body and osteoderms were dried to constant weight at 70 °C, then ashed in a muffle furnace at 650 °C overnight.

STANDARD METABOLIC RATE (SMR)

Oxygen consumption was measured in an open system using a Servomex OA 184 oxygen analyser in a constant temperature room (±1 °C), the output was recorded on a Honeywell Electronik 15 pen recorder. Lain (1979) and Davies (1982) describe in detail the measurement of SMR with this system.

The cylindrical animal chambers (51 × 10 or 30 × 7.5 cm diameter) allowed little room for activity, though the lizards could turn and coil naturally. A lizard, fasted for at least 5 days, was placed in the chamber in the evening. The chamber was then connected to an air supply, covered with a dark cloth, and left at the test temperature. Oxygen consumption was measured for 3–4 hr the following day, and the lowest stable rate corrected to STP ($\dot{V}O_2$) and taken as the SMR. Flow rates of 50–250 ml min^{-1} were used according to temperature. Resting $\dot{V}O_2$ was also measured at 11 °C during the third and sixth weeks of hibernation (HMR_3, HMR_6).

ACTIVE METABOLIC RATE (AMR)

The time course of $\dot{V}O_2$ during and after a bout of maximal activity was followed using a head mask made from a clear plastic syringe barrel with an added flexible rubber neck. Air was drawn through the syringe nozzle, and entered the mask via 2 m of tubing (to prevent exhaled gases escaping) let into the syringe barrel. The control air stream passed along a similar tube system. Exhaled air passed through 1 m of tubing, a cylinder of desiccant, and a flowmeter, total volume <200 ml. There was little mixing of gas along this system as the flushing half time was less than half a minute at flow rates of 250–350 ml min^{-1}.

A fasted lizard was rested overnight at the test temperature. The mask was placed over the head to the start of the lateral groove, taped in place, and $\dot{V}O_2$ measured for 1 hr. Two pin electrodes were placed in the base of the tail and the lizard was stimulated with 5 pulses s^{-1} of 50–80 V AC and 1 ms duration from a Palmer H45 stimulator for 10 min (Gratz & Hutchison, 1977). This was the minimum necessary to produce maximal activity, and was performed under licence (No. SWI 5115 and certificate A1). $\dot{V}O_2$ was measured for a further 2 hr. Mean $\dot{V}O_2$ was calculated for successive 3 1/3 min intervals from the pen record.

LACTATE PRODUCTION

Body lactate was measured in five lizards acclimated overnight to 25 °C; two resting and three after being stimulated to exhaustion as above. Each lizard was frozen in liquid nitrogen and stored in a deep freeze for up to 24 hr. The osteoderm coat was removed from the frozen lizard, which was then pulverized and homogenized in 4 times its weight of cold 0.9% NaCl saline in a cold room (2 °C) (modification of McClelland, pers. comm., to the method of Bennett & Licht, 1972). 60 ml of the homogenate was centrifuged at 3000 r.p.m. for 5 min, and 5 ml of the supernatant was deproteinized with 0.5 ml of 60–62% perchloric acid. This was centrifuged at 10 000 r.p.m. and 0 °C for

10 min, and 0.1 ml of the supernatant was analysed against duplicate lactate standards using an enzymatic test kit (UV method, BCL Cat. 124 842) and Unicam SP600 UV spectrophotometer. In calculation it was assumed that 1 g of body homogenized to 1 ml. Allowing for a small amount of muscle left in the tail tip after skinning, whole body lactate was expressed per g live weight.

RESULTS

THERMOREGULATION

Figure 1a shows the range of T_s present in the cages and the T_bs recorded. Maximum T_b was 35°C, though the hot end of the cages was much hotter, up to 60°C at the surface. Mean activity T_b was 27.7°C, SD 2.7°C, and the activity T_b range was 11°C (24–35°C) (Fig. 1b).

SMR

There was no significant difference between SMR of the different individuals—Friedman's method (Sokal & Rohlf, 1981) χ^2 test, $0.1 > P > 0.05$. Mean and SD of the untransformed rates are shown in Table 1. Mean coefficient of variation (CV = SD/$\bar{x}$, as a percentage) of the untransformed rates are shown in Table I. Mean CV = 18.5%.

The overall temperature sensitivity of SMR is described by least-squares regression of log SMR (μl g^{-1} h^{-1}) on temperature, T (°C);

$$\log \mathrm{SMR} = 0.0478\,T + 0.315$$

Overall Q_{10} (antilog of the slope expressed as SMR change per 10°C interval) is 3.01 (95% confidence limits 2.73 to 3.31, from SE of slope and *t* distribution).

HMR

There was no depression of $\dot{V}O_2$ at 11°C during hibernation, the rates (μl g^{-1} h^{-1}, SD) being;

SMR	5.3	1.4
HMR_3	5.3	1.0
HMR_6	5.3	0.6

AMR

When stimulated the lizards thrashed about energetically for ~15 seconds, then twisted and rolled over with decreasing vigour for up to 3 min, at all three temperatures. They did not ventilate during activity, and so the oxygen "consumed" decreased during the activity period (Fig. 2). At 35°C inhalation and heavy breathing followed immediately after activity ceased, but still within the period of stimulation. There were time lags between the end of activity and the start of breathing at 15 and 25°C (Table II).

As a realistic AMR could not be measured directly, I have instead calculated the maximum oxygen exchange capacity, as the maximum mean $\dot{V}O_2$ over any three or any two consecutive 3 1/3 min-intervals (Table II). Also shown is the total oxygen consumed during activity and recovery, estimated by extrapolating the curves to SMR at three hours post-stimulation (ignoring those affected by spontaneous activity).

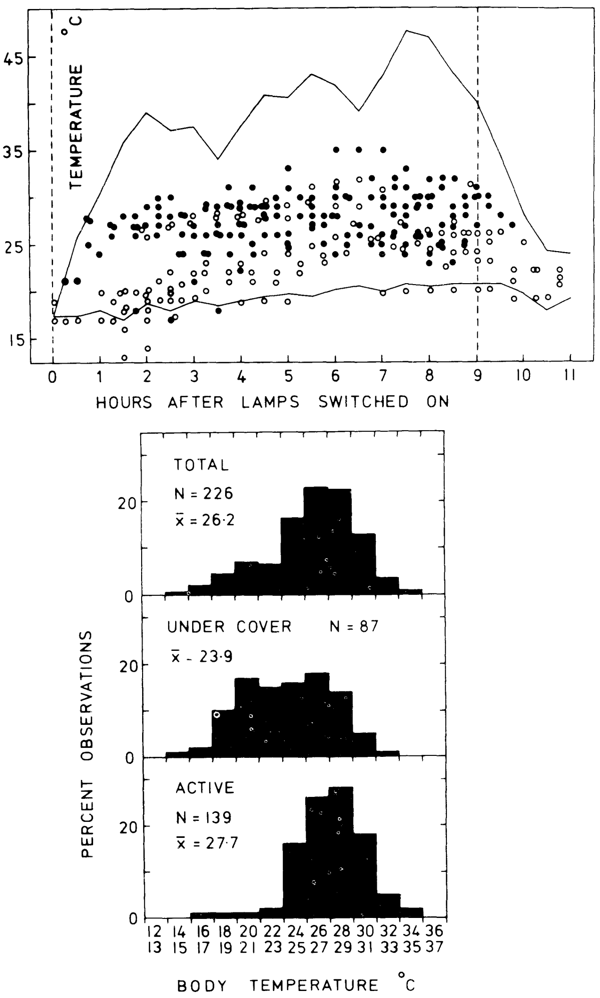

FIG. 1. Thermoregulation of *O. apodus* in a photothermal gradient. (a) Telemetered T_bs of active animals (●) and (○) animals inactive in the substrate. Heat lamps on for 9 h between the dashed lines. Solid lines are T_s at the hot and cold ends of the cages, mean values for the two cages on several days. (b) Frequency distribution of T_bs between 2–9 h after the heat lamps were switched on.

TABLE I. Standard metabolic rate of *O. apodus* at different temperatures (μl O_2 g^{-1} h^{-1})

	Temperature (°C)					
	11	15	20	25	30	35
$\bar{x}$	5.3	15.1	20.1	32.3	56.7	92.4
SD.	1.4	3.1	3.6	5.4	10.7	10.0

LACTATE PRODUCTION

Whole body lactate concentrations (mg g^{-1}) were;

resting	0.21, 0.38 ;	$\bar{x} = 0.29$
exhausted	0.82, 0.82, 0.88;	$\bar{x} = 0.84$

BODY COMPOSITION

Mean body composition ($n = 2$) was 63.5% water, 23.9% combustible matter, 9.1% osteodermal ash, and 3.5% other ash.

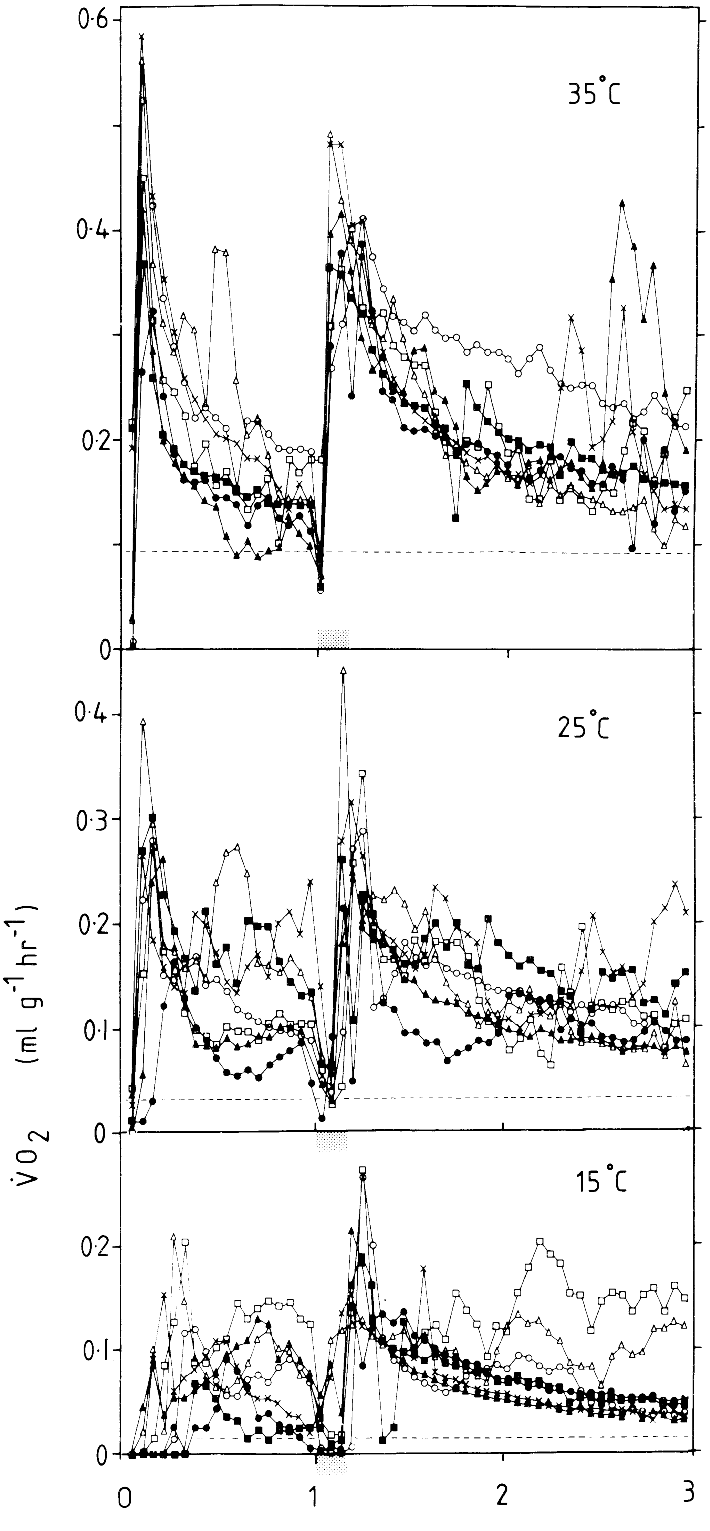

FIG. 2. Time course of oxygen consumption during maximal activity and recovery in *O. apodus* at three temperatures. Animals were fitted with a mask at 0 h, stimulated for 10 min from 1 h (shaded bar), and allowed to recover. Symbols identify the seven individuals. Dashed lines show SMR.

TABLE II. Duration of activity and apnea, and active metabolic rates of *O. apodus* at three temperatures. Means (with SD), $\dot{V}O_2$ in $\mu l\ g^{-1}\ h^{-1}$ unless otherwise stated. See text for derivation of metabolic parameters

	15°C	25°C	35°C
Duration of activity (min)	2–3	2–3	2–3
Duration of apnea (min)	7.7 (4.8)	6.5 (1.3)	3.0 (1.0)
$\dot{V}O_2$ during 10 min stimulation	39 (36)	109 (52)	284 (49)
Max 10 min $\dot{V}O_2$	161 (28)	238 (44)	387 (44)
Max $6\frac{2}{3}$ min $\dot{V}O_2$	180 (35)	267 (32)	421 (60)
Total $\dot{V}O_2$ in 3 h ($\mu l\ g^{-1}$)	155	295	520
$\dot{V}O_2$ cost ($\mu l\ g^{-1}$)	110	171	244

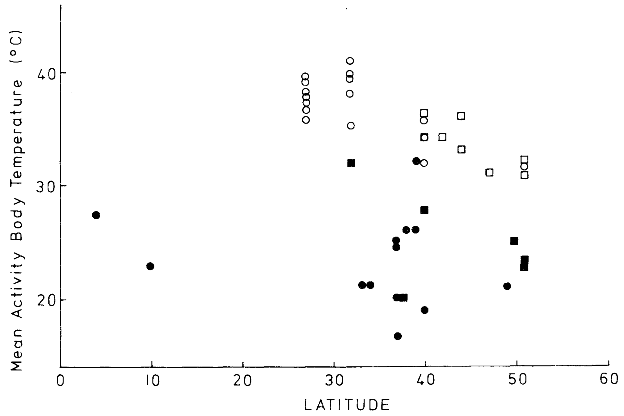

FIG. 3. Latitudinal variation of mean activity T_b in anguids (solid symbols) and lacertids (open symbols). Circles are data from the field or outdoor enclosures, squares are from laboratory gradients.

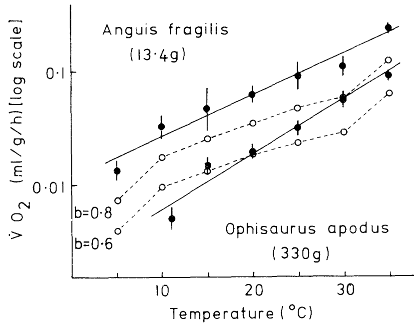

FIG. 4. SMR of male *O. apodus* ($n = 7$) and *A. fragilis* ($n = 5$, data from Patterson & Davies 1978a), semi-log plot. Dots are arithmetic means with 95% confidence limits of the mean (from log-transformed data). Solid lines are least square regression fits to the log-transformed data. Open circles and dashed lines show *A. fragilis* data extrapolated to 330 g using exponents of 0.8 and 0.6 in the relation $\dot{V}O_2 \propto \text{weight}^b$.

DISCUSSION

THERMOREGULATION OF ANGUIDS

The mean activity T_b found for *O. apodus*, 27.7°C, is rather low for a warm temperate lizard, and supports the observation that anguids as a group have low activity T_bs (Brattstrom, 1965; Avery, 1982). Figure 3 compares mean activity T_bs (including data from the field, outdoor enclosures, and thermal gradients) of anguids (including *Anniella*) with those of lacertids, a "typical" lizard group. The former includes data from all the references cited by Avery (1982), plus Saint-Girons & Saint-Girons (1956), and Patterson & Davies (unpublished) field T_b range and gradient mean and range for English *Anguis fragilis*. The latter are data from some of the publications listed in Avery's (1982) table IV, selected on the basis of accessibility, which are marked * in the reference list. Mean activity T_b of the anguids, 23.7°C ($n = 19$, SD $= 4.1$) is significantly lower than that of the lacertids, 35.6°C ($n = 24$, SD $=$ 2.9)—Wilcoxon two sample test (Sokal & Rohlf, 1981) $P < 0.001$.

SMR, THERMOREGULATION, AND LATITUDE

The SMR found in *O. apodus* is only 67% and 63% of that predicted from equations in Bennett & Dawson (1976) for a 330 g lizard at 20°C and 30°C, respectively. Reduced SMR has been found in other limbless lizards, by Withers (1981) and Kamel & Gatten (1983), but it is not clear whether this is a real difference or simply reflects more rigorous criteria of resting in these recent studies, compared to those on which Bennett and Dawson's equations were based. Stinner (1982) raised the same point in relation to SMR of snakes.

Figure 4 compares SMR in *O. apodus* with that of male *A. fragilis* from S. England (Patterson & Davies, 1978a). The latter were kept in a photothermal gradient against a background of 15°C ("summer acclimation"). These data have a similar degree of variability, $\overline{CV} = 18\%$. The overall temperature sensitivity is described by;

$$\log \text{SMR} = 0.0363\ T + 1.07$$

$Q_{10} = 2.31$ (95% CL: 2.11 – 2.52), significantly less than that for *O. apodus* (by non-overlap of CL).

The association of high latitudes with lower Q_{10} s within ecologically similar species pairs was also found by Patterson & Davies (unpublished, in Davies, Patterson & Bennett, 1981) and Davies & Bennett (1981). It makes functional sense if animals at high latitudes are normally active with a wider range of T_bs, and would give them a variability of performance similar to that of animals at low latitudes. This assumes that SMR and performance are correlated (see Taigen, 1983). The data available for anguids show no correlation between activity T_b range and latitude (Fig. 5), Spearman rank correlation $r_s = 0.088$, $P = 0.36$. The lacertids show a highly significant correlation, $r_s =$

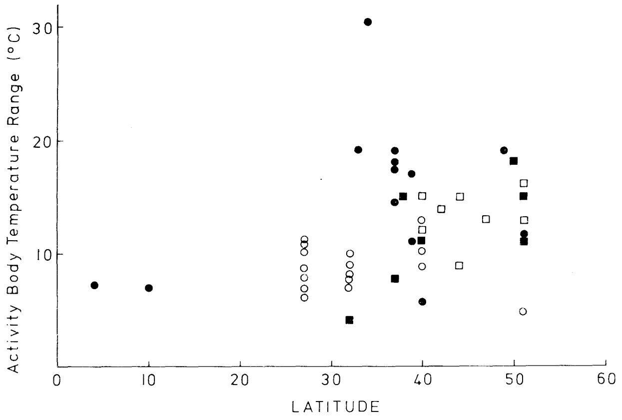

FIG. 5. Latitudinal variation in activity T_b range in anguids and lacertids, symbols as in Fig. 3. Ranges from Huey *et al.* (1977) were calculated as 4 times SD.

0.497, $P = 0.007$, though this is complicated by most of the low latitude populations being studied in the field, while those from high latitudes were studied in thermal gradients. Lacertids have significantly narrower T_b ranges ($\bar{x} = 10.5$°C, SD = 3.2) than anguids ($\bar{x} = 14.0$°C, SD = 6.3)—Wilcoxon two sample test $0.05 > P > 0.02$.

SMR of *A. fragilis* and *O. apodus* also differ significantly in elevation (Fig. 4). Much of this is probably due to the size difference, the slow worms being about 25 times smaller than the glass "snakes". Also shown in Fig. 4 are the slow worm data weight-corrected to 330 g using exponents of 0.8 and 0.6 in the relation SMR $\propto$ weight^b. The former is probably about right for lizards (Bennett & Dawson, 1976) and produces an elevation of *Anguis* metabolism at lower temperatures, as expected of a cool versus a warm temperate species (Aleksiuk 1971; Davies *et al.*, 1981). This makes functional sense if animals at high latitudes are normally active at lower T_b, and would give them a similar level of performance to animals at low latitudes (again assuming a correlation between SMR and performance). The anguid data overall do not support this hypothesis (Fig. 3, $r_s = 0.005$, $P = 0.49$), possibly because some of the low latitude populations studied are montane. The lacertid data show a strong correlation with latitude ($r_s = -0.77$, $P = 0.001$).

Reduced MR during hibernation is more likely in animals from high latitudes (e.g. Aleksiuk, 1976; Patterson & Davies, 1978b), where SMR is elevated as a compensation for low T_b. Although no depression of metabolism was found in *O. apodus*, the lizards appeared to show true hibernation behaviour (eyes closed, no movement about the cage or when handled) rather than merely cold-induced torpor (Patterson & Davies, 1978b). Hibernation occurs in the wild (Obst, 1981), and some captive individuals refuse food at the onset of winter (Couch, pers. comm.).

AMR AND LIFESTYLE

The short burst of activity on stimulation appears typical of escape behaviour in the wild (Obst, 1981), the lizard "can move fairly fast . . . but lacks stamina" (Arnold & Burton, 1978). When freshly caught, most individuals twist and roll briefly, although some lie still. It is likely that escape behaviour in the wild is apneic as well, possibly a result of the complete osteoderm coat with expansion limited to the lateral groove; movement and ventilation may be physically incompatible. The groove was folded during activity. This highlights the difficulty of measuring the use of oxygen in the tissues ("respiration" in the strict sense) from the reduction of oxygen in the expired gases (oxygen consumption) during short periods of activity. In the following discussion it is assumed that AMRs reported in the literature are the maximum observed rates of oxygen consumption, *i.e.* equivalent to oxygen exchange capacity.

Maximum oxygen exchange capacity of *O. apodus*, 0.4 ml g^{-1} h^{-1} at 35°C, is one of the lowest reported for lizards, similar to that of some of the larger skinks (Table II of Bennett, 1982). Scope for oxygen exchange is also low, 0.23 ml g^{-1} h^{-1} at 25°C, 0.50 ml $g^{-0.865}$ h^{-1} mass adjusted (Kamel & Gatten, 1983), compared to a mean rate of 0.71 ml $g^{-0.865}$ h^{-1} for 21 lizard species (Kamel & Gatten, 1983). Factorial scope was 11 times, 7.3 times, and 3.6 times SMR at 15, 25 and 35°C, respectively; Kamel & Gatten's mean for 21 lizard species at 25°C is 8.1 times SMR.

In correlation with this low aerobic support for activity, *O. apodus* is probably a slow moving predator (a cruising forager—Regal, 1983) which finds molluscs, large arthropods and occasional vertebrates in vegetation (Arnold & Burton, 1978; Obst, 1981). They are especially active during damp periods, observed by Arnold & Burton, (1978), but are also active in the hottest weather. The American *O. ventralis* presents an interesting contrast; Kamel & Gatten (1983) found a very high aerobic scope, 1.2 ml g^{-1} h^{-1} (mass adjusted 2.0 ml $g^{-0.865}$ h^{-1}, and factorial scope of 33 times) and note that this species is alert and active, behaviourally comparable to *Varanus* and *Cnemidophorus*. *Anniella* and the amphisbaenian *Trogonophis* have similar mass adjusted aerobic scopes to *O. apodus* (0.52 and 0.66 ml $g^{-0.865}$ h^{-1} respectively) and are also relatively inactive (Kamel & Gatten, 1983). They noted periods of apnea during activity in *Trogonophis.*

ANAEROBIOSIS AND PREDATOR DEFENCE

Apart from reptiles which must subdue relatively large prey (Feder & Arnold, 1982), anaerobic metabolism is probably used infrequently, when escaping from a predator (Bennett, 1982). It is unsustainable, and leaves the animal exhausted with a high lactate load, 0.55 mg g^{-1} above resting in *O. apodus* at 25°C. This is lower than other lizards (Table X in Bennett, 1982). At 25°C *Anniella* and *Trogonophis* both produced 0.84 mg g^{-1} and *O. ventralis* produced 1.9 mg g^{-1} during two minutes of activity (Kamel & Gatten, 1983).

Although ready to take flight and to thrash briefly if captured (particularly when hot, pers. obs.), *O. apodus* has a heavy reliance on armour for defence. The osteoderm coat is flexible but complete (Elkan, 1976), and is very difficult to pierce or cut. As a comparison, a 12 cm *Testudo* shell (live weight ~360 g from data in Stubbs, Hailey, Tyler & Pulford, 1981) was burnt at

650°C, and yielded 44.4 g ash. An index of mineral defences given by the ratio

$$\frac{\text{weight of armour}}{\text{weight of body less armour}}$$

is 0.14 for this shell and 0.10 for *O. apodus*, *i.e. O. apodus* is 70% as armoured as the tortoise. The German name "panzerschleiche" is considerably more appropriate than glass lizard or snake—*O. apodus* cannot autotomize, having no fracture planes in its tail vertebrae (Etheridge, 1967).

The genus *Ophisaurus* presents a variety of lifestyles and escape behaviour. The small American *O. attenuatus* and *O. ventralis* are active lizards, seldom burrowing, which make strenuous effort to escape and free themselves (Conant, 1975; Kamel & Gatten, 1983), and readily shed their tails (H. M. Smith, 1946), though the similar *O. compressus* has no autotomy planes in its vertebrae (Etheridge, 1967; Conant, 1975). The Asian *O. gracilis* and *O. harti* are larger, forest dwellers, found under logs and stones, active at dusk, sluggish, and do not automonize readily. They are physically similar to *O. apodus*. *O. gracilis* shams dead when handled (M. A. Smith, 1935).

The 0.55 mg g^{-1} lactate produced by *O. apodus* during activity would require 0.10 ml O_2 g^{-1} for removal by glucose resynthesis if the cost is met by oxidation of fatty acids (Gratz & Hutchison, 1977), amply covered by the observed 0.17 ml O_2 g^{-1} cost of activity above SMR (Table II). This oxygen cost of activity had a low Q_{10}, 1.5 between 15–25°C and 1.4 between 25–35°C, as does the amount of lactate produced at exhaustion in a number of reptiles (Bennett, 1982).

It may be mentioned finally that the seven males used in this study were the only glass lizards caught; the probability of such an extreme sex ratio in a random sample from a series of encounters of even sex ratio is 0.016 ($= 2 \times 0.5^7$). This may be due to some difference in behaviour between the sexes in summer. Female *O. ventralis* (Noble & Mason, 1933, Vinegar, 1968) and *O. harti* (Pope, 1956) brood their eggs.

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POSSIBLE ORIGINS OF IRISH NATTERJACK TOADS (*BUFO CALAMITA*)

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SUMMARY

Three routes by which natterjack toads could have arrived at their existing Kerry strongholds in Ireland are discussed. These are: (1) Accidental or deliberate introduction by Man; (2) Migration from England via Wales or Scotland while land bridges persisted after the end of the last ice age; (3) Migration from the Bay of Biscay region directly to the Kerry area along the probable coastline of the late glacial or early postglacial period. The latter route, which would have been quite separate from that followed by natterjacks entering mainland Britain, is considered to be the most probable.

INTRODUCTION

The natterjack toad *Bufo calamita* is widely distributed in Europe, being most abundant in Iberia but becoming increasingly localized northwards and eastwards from this stronghold; it is excluded from much of southern Europe by the Alps and the Carpathians, but reaches as far east as Baltic Russia and as far north as Scotland and Sweden (Beebee, 1979). The recent discovery of two small colonies in Austria (Gollmann & Tiedemann, 1980) means that the species is presently extant in seventeen countries.

Almost certainly the only successful refuge for the natterjack, in common with some other European amphibia such as the marbled newt *Triturus marmoratus,* during the last ice age was the Iberian peninsula (Beebee, 1983). From this locality it must have spread, following the retreating ice, to something like its present distribution limits. Since sea levels were lower in this period (8–10 000 years BP (= Before Present)) than they are now, there would have been no serious obstacle to amphibians reaching, for example, Britain and Scandinavia until the sea levels rose to cut them off 7–8000 years BP.

There is, however, at least one problem with this simple view of events. Natterjack toads were discovered in County Kerry, south-west Ireland, nearly 200 years ago and reported sometime later by Mackay (1836). The arrival of *Bufo calamita* in this particular area is not so easy to fit into the general pattern of colonization for reasons which have long been debated, and which are reconsidered and expanded upon in this paper.

HYPOTHESES

It seems to me that there are three main possible explanations for the arrival of natterjacks in County Kerry. These are:

(1) The introduction, deliberate or accidental, of toads to the area by Man within historical times.

(2) The contraction of a once much wider range in Britain and Ireland, the toads having reached Ireland via a land bridge with Britain, such that by chance the species has persisted in Ireland only in the south-west.

(3) The natural colonization of Kerry by a route quite separate from that taken by natterjacks in the remainder of Britain.

These three options will now be considered in turn in greater detail, in the light of existing evidence relevant to each.

(1) Several writers have alluded to the possibility that natterjacks were introduced to Ireland by human agency, apparently in relation to an old local legend that some were once released from a ship at the head of Dingle Bay (Smith, 1951). Certainly no positive evidence, in the form of any records, of such a deliberate introduction has ever come to light and a motive would be difficult to imagine. Another idea, mentioned most recently by Frazer (1983), is that an accidental introduction may have occurred; the story goes that ships carrying sand ballast picked up from, say, the Liverpool Bay area where natterjacks are common may have inadvertently collected some toads with it and later discharged them all together in the Dingle Bay region. Colonization of the local prime habitat would have followed rapidly.

Apart once again from the lack of any specific evidence supporting this notion, there seem to be at least two objections to it as a likely history of natterjacks in Ireland. Firstly, in an area so well endowed with sandy margins as Dingle Bay it is surely unlikely that sand ballast would have been unloaded onto land, with all the effort this would entail, when the material would have no local value. The most expedient procedure would have been to dump the ballast overboard. Unfortunately adult natterjacks are killed quite rapidly when immersed in seawater, as this would have entailed, rendering survival for colonization very unlikely (Mathias, 1971, and general observations of seawater inundation effects at natterjack breeding sites). A second problem relates to the recent distribution of *Bufo calamita* in Kerry when this is considered in detail. Although it could be argued that a single release anywhere around the main basin of Dingle Bay could have resulted in the toads spreading from Rossbeigh round to Inch because there is (or was) virtually continuous suitable habitat, the presence of natterjacks in some of the more outlying Kerry sites is not so readily explained. Most of the historical records do hail from this main Dingle Bay region, but natterjacks were recently discovered in large numbers on a sand dune system on the north side of the Dingle peninsula and separated from Dingle Bay by 15–20 km of upland terrain, with altitudes up to 500 m between the toad colonies (Gresson & O'Dubhda, 1971). The lack of historical records from the northern site could mean that natterjacks only reached there recently with help from Man, but it is equally possible that they have long been present but unrecorded. Even in more densely populated England, new natterjack sites were still being discovered in 1983. There are also older natterjack records from further west around the Waterville peninsula south of Dingle Bay, notably Ballycarbery (Valentia), Coomakista and Caherdaniel (Gresson & O'Dubhda, 1974). The first of these was verified by a visit to the landowner to have had natterjacks up until around 1945–1950, when the breeding ponds were drained as an agricultural improvement; records from the other two are less certain, though at least one has apparently suitable habitat. The point is that even Ballycarbery is separated from Dingle Bay by more than 30 km of mountainous coastline, the other two sites being even further away. Any natural spread of natterjacks to these areas from Dingle Bay is difficult to explain in light of the highly unsuitable habitat blocking their way, and it seems that at least 3 separate introductions must be invoked to explain the recent distribution of natterjacks in Ireland. Furthermore, there is the added coincidence that the introductions were all in the same area although potentially suitable dunes exist elsewhere in Ireland. One of these, in County Wexford, has even been considered recently for a deliberate introduction experiment.

(2) Natterjacks may have reached Ireland via mainland Britain and the various land bridges which existed in early post-glacial times between England and France, and Wales (or Scotland) and Ireland. In this model, *Bufo calamita* must have been quite widely distributed for a while in Ireland, at least in the south, as it must have been in England probably in the very early postglacial period before forest cover became dense (Yalden, 1980). Changing environmental circumstances, most notably afforestation, then precipitated a contraction in range with the Kerry dunes being the only suitable habitat to survive over the entire intervening millenia. Also of some relevance here are the other so-called lusitanian fauna and flora, species of animals and plants generally characteristic of the Iberian area but also found in some south-western parts of Britain and notably County Kerry (e.g. the Kerry slug, *Geomalacus maculosus*). Many authors have drawn attention to this feature of distribution (e.g. Matthews, 1955), some even suggesting that there was an ice age refuge somewhere off the south-west coast of Ireland which later acted as a colonization source for these species.

There are a number of objections to explaining natterjacks in Kerry on the basis of any of these ideas and observations. Firstly, Ireland separated from mainland Britain earlier than Britain separated from France as the sea levels rose, and this is a commonly invoked explanation of the paucity of herpetofauna in Ireland (Smith, 1951). Natterjacks would have had to have spread more rapidly than most other amphibian species for colonization to have been achieved in this way, using circuitous routes through France, England

and Wales or Scotland to reach Ireland. Apart from *Bufo calamita*, the only other amphibians found in Ireland are the smooth newt *Triturus vulgaris* and the common frog *Rana temporaria;* the latter may have been deliberately introduced in the seventeenth century, though it could have been native before that time (Smith, 1951). Both of these species are much more cold-tolerant than the natterjack, and would have been expected to follow the retreating ice more closely than the latter. Indeed, the other British species *Bufo bufo*, *Triturus helveticus* and *T. cristatus* are also more cold-tolerant than natterjacks yet they never established themselves in Ireland.

This problem is further compounded by the historical distribution of natterjacks in Britain. The most direct colonization route would presumably have involved south-west England or, more probably, south Wales but these are two regions which have never yielded reliable records for the species despite the existence of sand dune habitats there (Beebee, 1976; 1977; 1978). If these areas did provide colonization routes, no sign of them has persisted in the recent distribution. Natterjacks were present on the eastern North Wales coast until the 1940s and still on the Scottish coast of Solway, but in neither case are there any good records from the more westerly dune systems that exist along these coastlines; again, expected links are missing. Dunes in all of these regions are often separated by mountainous intrusions, and this is probably enough to explain the absence of natterjacks in many cases, but it also casts doubt on their historical value as colonization routes.

An ice age refuge off the west coast of Ireland seems an unlikely explanation and has been largely discredited by students of lusitanian species. Even if an offshore area escaped coverage by ice, its close proximity would have created tundra conditions totally unsuitable for natterjacks. Although the mild winters typical of County Kerry may be of present importance to some of the lusitanian species found there, especially plants with little frost tolerance such as the strawberry tree *Arbutus unedo*, they can have little significance for *Bufo calamita*. Natterjacks survive perfectly well in the intensively cold winters of central Europe; it is warm summers that this animal needs, something for which Kerry is not noted. Past and present climatic features seem unlikely to explain the survival of natterjacks in south-west Ireland in any classical lusitanian context.

(3) A third way in which *Bufo calamita* might have arrived in south-west Ireland does not involve passage through any other part of Britain. Figure 1 outlines the south-west approaches to Britain and also marks the 100 metre sea-depth contour. During the height of the last glaciation, sea levels certainly fell to around or below this line; the deeper channel between Pembroke and Wexford (St George's channel area) is thought to mark an ancient outflowing from the Irish sea during this period. The exact situation at the end of the ice age is not totally clear, but there is widespread belief that for some time the 100 m contour reflected the approximate land edge (Kidson *et al.*, 1977).

This coast, with its south-westerly aspect and substantial estuaries, would seem ideally placed for dune formation and it seems quite possible that sandhills may have been a major feature between Brittany and Kerry. Such dunes would, of course, be prime natterjack habitat. Estuaries such as the old St George's Channel bed would probably not be a serious hindrance, especially if sandy, the situation perhaps being similar to Solway over which natterjecks have clearly been able to traverse. It is not even difficult to imagine mechanisms for crossing such obstacles, such as an altered outflow pattern in the estuary transferring land continuity from one side to another. If a route of this kind existed, the surviving contours indicate that it would lead quite directly from Brittany (where natterjacks are still abundant in coastal dunes) to south-west Ireland.

However, current evidence suggests that following the retreat of the glaciers the initial rise in sea level (especially between 10 000 and 9000 BP) was quite rapid, as measured in areas such as the Somerset levels where concurrent land uplift was not a complicating factor (Kidson *et al.*, 1977). Would there have been time for natterjacks to use this route? In part this depends upon where exactly natterjacks were starting from at the end of the Younger Dryas period and the start of the final warming 10 000 years BP. Recent evidence confirms that an initial warming, judged by the disappearance from Britain of a number of Arctic insects (e.g. the Byrrhid beetle *Simplocaria metallica*), took place about 13 000 BP and led to the disappearance of most, if not all glaciation from Britain south of Scotland. However, almost exactly 1000 years later the climate deteriorated again and thermophilic beetles which had already managed to establish themselves (e.g. the carabid *Bembidion octomaculatum*) were once again exterminated. Some glaciers probably reappeared in the Lake District and North Wales, but it seems that this relapse was not to a period of cold comparable with that which had gone before; although some Arctic/Alpine beetles reappeared, the spread of birch woodland in England continued through to the final warming some 2000 years later (Neale & Flenley, 1981). A possible scenario, therefore, is that natterjacks moved some way northwards along the Atlantic coast during the initial warming and were not forced all the way back again to Spain during the Younger Dryas deterioration. The ameliorating effect of the adjacent Atlantic Ocean, with the developing Gulf Stream, could have maintained the toads perhaps as far north as Brittany. They would in that case have had perhaps 5–600 years to spread a comparable number of kilometres to the vicinity of Kerry before the old coast was inundated, and a rate of 1 km year^{-1} seems quite a reasonable one for such an animal; individual adult natterjacks have been found to wander more than 1 km in the course of a single summer, and the species must have crossed central Europe at a fair pace to have made the much more distant crossing to Sweden before the Kattegat opened up.

DISCUSSION

It is unlikely that the origins of Irish natterjacks will ever be established unequivocally, but it is of some interest to try and assess the most probable history of events. Accidental or deliberate introduction certainly cannot be ruled out; for example, a single initial introduction could have been followed by subsequent translocations to nearby but isolated localities in the form of spawn on wading birds' feet, or some such mechanism. Nevertheless it seems to me that introduction is an unlikely cause, relying as it does on a peculiar series of low probability events.

If *Bufo calamita* ever was more widespread in Ireland than is presently the case, it is perhaps surprising that some fossil evidence has not emerged by now. Since there are no other bufonids in the country, interpretation of such evidence should be a relatively easy matter. Furthermore, there has to be some kind of special pleading to explain the absence of natterjacks from other dune systems in southern Ireland.

Of the three options considered, an old coastal corridor route between Brittany and Kerry seems to me the most probable on the basis of existing evidence. It explains quite nicely why, in Ireland, only Kerry has natterjack populations; it is simply the first surviving stop along this corridor, and it is quite striking that, in this part of Ireland, virtually all suitable areas as far north as the north Dingle peninsula have yielded records of the species. The surviving natterjack distribution can also be interpreted to infer that there was indeed only a very limited time over which this route was possible; natterjacks have not been found further north along the west coast of Ireland, nor in south-west England, perhaps because rapidly rising seas obliterated the old coast too quickly for further spread. Retreating into Dingle Bay and being stranded in the various sandy sites would have meant moving no more than 60–65 km from the old coast, whereas the nearest possible habitat in Cornwall and Wales was 100–150 km away (Fig. 1). These different distances mean that, during the early rapid rises in sea level, the coast would have been receding at only 30–40 metres year^{-1} near Kerry but at some 100 metres year^{-1} in the more gradually shelving regions west of the Bristol Channel. This in turn may explain why natterjacks from these central regions of the old coast did not survive to colonize more easterly parts of southern Ireland, south Wales or south-west England; the inundation was simply too fast for them to cope with. The rocky nature of coastal regions surrounding Kerry would have effectively prevented any spread of natterjacks out of the area once sea levels had risen to their present height. Colonization of Kerry by lusitanian species using a direct Iberia-Ireland route has also been hypothesized by Yalden (1982).

This hypothetical coast dune route may also explain the colonization of Ireland by its other two amphibian species. *Rana temporaria* and *Triturus vulgaris* are, apart from *Bufo calamita*, the British amphibians found in greatest abundance in sand dune habitats throughout the country. Although *Bufo bufo*, *Triturus helveticus* and *T. cristatus* all do occur on dunes in some places, their numbers are usually very much lower (except, in the case of common toads, where recent afforestation has occurred) and they would not be expected to progress so fast along a dune-dominated coast. On this basis, it is suggested that frogs may be truly native to

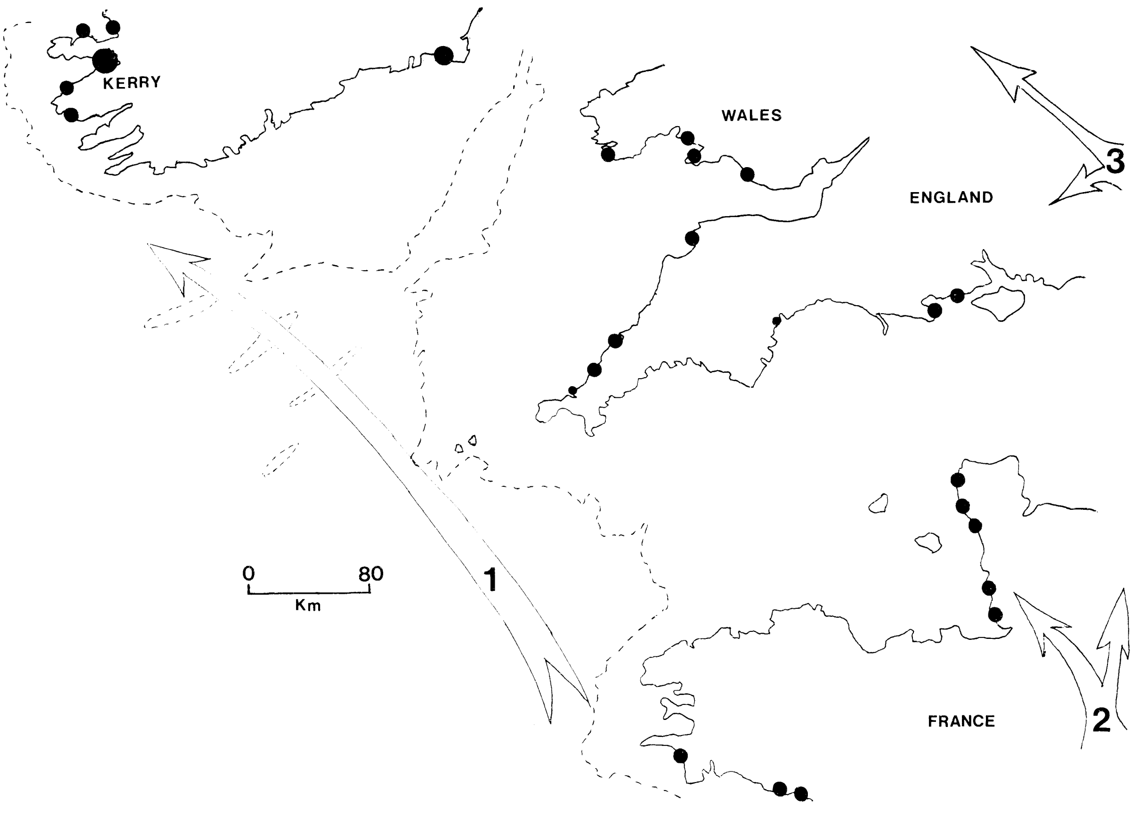

Fig. 1. Geography of the south-west approaches to Britain. Solid line shows the existing coastline, dotted line the 100 m sea depth contour. Black dots show areas with extensive dune or otherwise sandy coastlines. Arrows indicate possible routes of natterjack toad movement in postglacial times: 1. Brittany-Kerry passage; 2. route to general north French coast; 3. route into mainland Britain, from more easterly land connection.

Ireland. Even Ireland's only reptile, *Lacerta vivipara*, might have arrived this way; common lizards do well on dunes and would move north faster than the more thermophilic sand lizards. Grass snakes, adders and slow-worms do inhabit dunes but much more rarely and in general the habitat seems less than ideally suited to them.

If the south-west corridor existed, the implication is that natterjacks in Kerry have different origins from those in Britain which probably arrived via a more easterly land bridge between Dover and East Anglia. This poses the possibility of looking at various morphological and biochemical characters to try and test for genetic differences between populations, which might in turn act as markers giving clues to their evolutionary histories. In other words, these hypotheses might be at least partially testable. Such differences have already been measured between natterjacks in Germany and Spain (Flindt & Hemmer, 1972), and between natterjacks on Swedish islands and those on the mainland (Andren & Nilson, 1983).

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ANATOMICAL ORGANIZATION OF ADIPOSE TISSUE IN CHELONIANS

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SUMMARY

1. Adipose tissue from eleven anatomically defined sites has been studied in 14 adult chelonians representing eight different genera.

2. The specimens ranged from well-fed individuals, which had mean adipocyte volume of 0.93 nl, to lean specimens of mean adipocyte volume less than 0.1 nl.

3. The volume of adipocytes from each of the eleven sites was determined separately.

4. The correlation between left-right homologous pairs of sites was greater than the correlation between non-homologous pairs of sites, but no consistent pattern of arrangement of adipocytes of different relative sizes was found.

5. Although the gross organization of chelonian adipose tissue is similar in several respects to that of mammals, the reptilian tissue lacks the organization at the cell level which has been found in all mammals studied.

6. The data therefore support the suggestion that anatomical organization of adipose tissue at the cell level is a special feature of mammals.

7. The data show that biopsies taken from any adipose deposit would be equally satisfactory for longitudinal studies of fat reserves in living chelonians.

INTRODUCTION

Although the ecology and physiology of lipid storage in reptiles has attracted considerable attention (Derickson, 1976), there has been little interest in the anatomical organization of reptilian adipose tissue since the work of Butler (1889) on the homologies of fat bodies in various reptiles. Frye (1981) stated that the microscopic structure of reptilian adipocytes was "essentially identical" to that of mammals. We decided to examine the anatomical organization of reptilian adipose tissue following the demonstration of a consistent pattern of cellular organization in mammalian adipose tissue (Pond and Mattacks, 1984). In these studies, the gross weight and mean adipocyte volume of numerous anatomically defined adipose deposits were measured in a variety of wild and captive mammals; a consistent pattern of distribution of adipocytes of different relative volumes was found in all species studied, regardless of body size or the fatness of the specimen. Species of widely different body conformation, such as camel, lion, marmoset or hamster differed mainly in the relative abundance of adipocytes at the various adipose deposits, not in the relative sizes of the adipocytes at these sites. Pond (1984) suggested that this organization of adipocytes in fatty tissue was a special feature of mammals, perhaps related to the evolution of energetically demanding activities which involve rapid or extensive accumulation and depletion of fat reserves. An obvious test of this hypothesis is to look for similar patterns of cell arrangement in other groups of vertebrates. Unfortunately, the adipose tissue of most non-mammalian tetrapods is concentrated in one or a few deposits, and even closely related genera differ greatly in the anatomical location of their adipose tissue (Pond, 1978).

Chelonians are one of the few groups to have the adipose mass dispersed between many sites, some of which are near dermal tissues, around limbs or between muscles. The gross organization of chelonian adipose tissue is thus similar in many ways to that of mammals; a study of its organization at the cellular level affords an excellent opportunity to determine whether the mammalian pattern of distribution of adipocytes of different sizes represents a special feature of mammals, as suggested by Pond (1984), or is simply a consequence of the fact that much of the adipose tissue in mammals is in superficial sites around or between muscles.

MATERIALS AND METHODS

In the interests of conservation of wild reptiles, no specimens were killed solely for this project. The specimens used died from various causes in CMP's personal collection, which was purchased or legally imported into the U.S.A. between 1975–1978 and into the U.K. in 1979. Terrestrial species ate boiled rice, meat, fruit, earthworms, cabbage and carrot. They were kept in an indoor pen of area 3 m^2, lit by fluorescent "sunlight" lamps. Aquatic species lived in a large tank similarly lit, and ate fish, worms and vegetables. Some specimens were preserved in formalin shortly after death and later transferred to 70% alcohol. Of more than 30 animals examined, 14 were found to have sufficient adipose tissue for analysis. Table I shows details of these specimens. They all had adipose tissue at each of the eleven sites listed in Table II; some also had fat at other sites, particularly anterior and ventral to the pectoral girdle and inside the abdominal cavity, but the presence of adipose tissue at these sites was not consistent enough for it to be included in the analysis. The mean adipocyte volume was determined for each site separately by measuring the diameter of about 40 cells, according to the methods described in Pond, Mattacks & Sadler (1984).

RESULTS

Because of the wide variation in the mass of the shell, it is meaningless to express fatness as the proportion of adipose tissue in the body. Instead, the mean volume of all adipocytes measured from each specimen is given in Table I. Experimental studies on laboratory rodents (Hirsch & Han, 1969) have shown that expansion or depletion of a constant population of adipocytes is the chief mechanism by which adipose tissue responds to changes in nutritional status. Therefore, an overall mean adipocyte volume is a rough measure of the relative fatness of the specimens; it shows that our sample includes both very well nourished and very lean specimens.

In order to detect any differences in adipocyte volume which are specific to certain anatomical sites, the same data are arranged according to anatomical site in Table III. Taking all fourteen specimens together, there were no significant differences in the volume of adipocytes at different sites. The trend towards larger cells in the three "groin" sites was noted in only five of the fourteen specimens, and for the group as a whole, the adipocytes at these sites were not significantly different ($P < 0.05$) from those elsewhere on the body. Similar data from mammals of widely different fatnesses clearly reveal site-specific differences in the relative sizes of adipocytes (Pond, 1984).

The correlation coefficients between the volumes of adipocytes at the five left-right homologous pairs of sites were significantly higher (Fisher's z-transform test, $P < 0.05$), than the correlation coefficients between the volumes of adipocytes from non-homologous sites. However, all the correlation coefficients were statistically significant ($P < 0.05$): there was no evidence for any site or sites containing adipocytes the volume of which failed to correlate significantly with those elsewhere in the same animal, as was found by Pond *et al.* (1984) for adipocytes in the "groin" sites of *ad lib.* fed sedentary guinea pigs. Like these guinea pigs, most of the chelonians used for this study had been in captivity for some time, but there was no evidence that the *ad lib.* diet and lack of strenuous exercise had

TABLE I. Particulars of chelonians used in the study

				Specimen-mean adipocyte volume	
Species	Sex	Weight (kg)	Cause of death	Mean (nl)	SD
Geochelone carbonaria	M	1.63	Perforated stomach/intestinal black	0.836	0.157
Geochelone denticulata	F	3.48	Abdominal congestion due to failure to lay mature eggs	0.485	0.155
Geochelone elongata	F	1.91	Respiratory infection	0.537	0.075
Testudo graeca	F	1.1	Nematode infestation	0.502	0.079
Testudo graeca	F	0.86	Inanition/respiratory infection	0.072	0.017
Cuora amboiensis	F	1.01	Pneumonia	0.931	0.145
Cuora amboiensis	M	0.65	Respiratory infection	0.780	0.118
Cuora amboiensis	M	0.56	Respiratory infection/old age	0.136	0.032
Clemmys insculpta	M	1.07	Respiratory infection/old age	0.327	0.054
Geoemyda pulcherrima	F	0.51	? old age	0.706	0.113
Pseudemys scripta elegans	F	0.81	Acute intestinal infection	0.636	0.069
Pseudemys scripta elegans	M	0.23	Unknown	0.530	0.210
Graptemys geographica	F	0.37	Nutritional deficiency	0.514	0.090
Podocnemis unifilis	F	0.69	Drowned	0.434	0.040

TABLE II. Anatomical location of the adipose tissue deposits studied

Name of site	Description
Behind foreleg	Fat posterior and ventral to forelimb, extending from pectoral girdle to carapace
Between shoulder muscles	Fat ventral to pectoral musculature and dorsal to inner surface of plastron
Anterior edge of shell	Superficial fat on inner surface of junction between plastron and carapace, on anterior half of body
Posterior edge of shell	As above, but extending over posterior part of body
Side groin	Fat anterior and dorsal to the muscles over femur, extending from pelvic girdle to knee
Ventral groin	Medial superficial fat deposit anterior and ventral to the pelvic girdle extending to inner surface of plastron

TABLE III. The mean volume (nl) of adipocytes from 11 different adipose deposits. The data are the mean of all 14 specimens listed in Table I.

Name of deposit	Mean adipocyte volume (nl)	SD
Behind foreleg, right	0.513	0.240
Behind foreleg, left	0.514	0.243
Between shoulder muscles, right	0.455	0.228
Between shoulder muscles, left	0.449	0.200
Anterior edge of shell, right	0.510	0.288
Anterior edge of shell, left	0.511	0.267
Posterior edge of shell, right	0.489	0.226
Posterior edge of shell, left	0.467	0.208
Side groin, right	0.630	0.301
Side groin, left	0.637	0.296
Ventral groin	0.634	0.310

promoted changes in the relationship of the volumes of adipocytes in different anatomical sites, as happened in the guinea pig (Pond *et al.*, 1984). However, the fact that adipocytes in homogous sites on the two sides of the body are more similar to each other than to adipocytes from non-homologous sites suggests that, as in mammals, there are locally acting determinants of adipocyte volume. These local factors play a relatively minor role compared to centrally controlled, systemic regulators of adipocyte volume.

DISCUSSION AND CONCLUSIONS

The mean volume of chelonian adipocytes falls within the range of those found in various species of wild and captive mammals by Pond (1984). The high correlation between independently measured left-right homologous pairs suggests that our preservation methods have not caused major changes in the volume of the adipocytes. Our methods should therefore be adequate to demonstrate a consistent pattern of anatomical organization of adipocytes, if one exists. We cannot find any clear pattern either within or between species. The fact that some of the specimens were elderly and had been in captivity for some time is unlikely to obscure any pattern which would be present in young, active animals. Although Pond *et al.* (1984) found that the relationship between the volume of adipocytes at different sites could be modified by changes in diet and exercise regime, the typical mammalian pattern of adipocyte organization could be seen in all specimens examined by Pond and Mattacks (1984), regardless of age or duration of captivity.

We conclude, therefore, that although the gross arrangement of chelonian adipose tissue is similar in some ways to that of mammals, the reptilian tissue lacks the organization at the cell level which has been found in both eutherian and metatherian mammals (Pond, 1984). These data thus confirm the suggestion that a consistent pattern of distribution of adipocytes of different relative sizes is a special feature of mammals, and is not necessarily a direct consequence of the fact that the adipose tissue is dispersed in superficial sites around limbs and in association with skeletal muscles. The data also indicate that biopsies taken from any adipose site would be equally satisfactory for monitoring changes in fat reserves in living reptiles.

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INTER-SPECIFIC COMPARISONS OF GROWTH RELATIONSHIPS IN CHELONIANS

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SUMMARY

Body length, mass, and volume were measured for certain chelonians. Inter-specific comparisons of length-mass and length-volume relationships showed a similar concomitant increase in the paired parameters for all species examined. Comparisons made to similar data in the literature supported the premise of a common growth coefficient representative of all chelonians.

INTRODUCTION

One expects an inherent relationship between body mass and size during the development of an organism. Meek (1982) reported on the allometric relationship between carapace length and mass in four chelonian species. A general equation representing the relationship for all the species showed carapace length proportional to body mass$^{0.34}$. Mosimann (1958) examined the inter-relationship of various measurements in *Chrysemys picta*, *Graptemys geographica*, and *Sternotherus odoratus*. Mass-length comparisons were also examined for *Chelydra serpentina* (Lagler & Applegate, 1943) and *Trionyx spinifer* (Dunson, 1967). Length-mass relationships were examined for snakes (Kaufman & Gibbons, 1975) and for the desert iguana, *Dipsosaurus dorsalis* (Minnich, 1971).

Described length-mass relationships are useful in understanding growth patterns for intra- and inter-specific comparisons. The purpose of this paper is to present allometric data on chelonians not previously examined. Length-mass relationships were examined for two species. Additionally, carapacal length-volume relationships were examined for four species representing a wide range of body forms. Comparisons are made to data of this type in the literature (Lagler & Applegate, 1943; Mosimann, 1958; Dunson, 1967; Meek, 1982). Generalizations are deduced on inter-specific shell variation.

MATERIALS AND METHODS

Data on carapacal length (mm) and mass (g) of *Terrapene ornata* (132.0–429.9 g) and *Kinosternon* *flavescens* (20.8–675.4 g) were obtained from field-collected individuals. Carapace length was determined by measuring the straight horizontal distance from the precentral to postcentral laminae. Mass was measured between 24–48 hr of capture. Carapacal length (mm) and volumetric (ml) data were obtained for *Chrysemys scripta elegans* (33–232 mm), *Kinosternon flavescens* (32–155 mm), *Terrapene ornata* (48–130 mm), and *Trionyx spinifer* (41–335 mm) from preserved specimens in the collection of the Texas Tech University Museum, Lubbock, Texas. Volumetric determinations were obtained by displacement in water.

Least-squares regression analyses were performed on the logarithmic-transformed data using the Statistical Analysis Systems package (SAS Institute Inc., 1982). Allometric equations are in the form $y = ax^b$. Analysis of covariance procedures in the SAS package were utilized to examine inter-specific comparisons in the two data sets. The t distribution was used to assign 95% confidence limits to the exponent in the allometric equations. Inter-specific variation was deemed significant at $P < 0{\cdot}05$. Comparisons to corresponding allometric equations in the literature were made using the confidence placed on the exponent b.

RESULTS

LENGTH-MASS RELATIONSHIPS

The allometric equation, $y = ax^b$, represents carapacal length (y) as the dependent variable, a = the intercept and b an exponent of mass (x). Indicated for each equation is the 95% confidence interval about b, the correlation coefficient (r) and the number of observations (n).

Allometric equations were as follows:

Kinosternon flavescens
$y = 15.65\,x^{0.354 \pm 0.009}$ $r = 0.994,\ n = 69$
Terrapene ornata
$y = 18.96\,x^{0.318 \pm 0.045}$ $r = 0.982,\ n = 9$

The inter-specific comparison of the length-mass data was not significant ($P > 0.05$).

LENGTH-VOLUME RELATIONSHIPS

Allometric equations presented here are as previously described for the length-mass comparisons; however, the dependent variable (y) = carapacal length and the independent variable (x) = volume. The derived equations were as follows:

Kinosternon flavescens
$y = 13.16\,x^{0.379 \pm 0.014}$ $r = 0.996,\ n = 24$
Chrysemys scripta elegans
$y = 15.99\,x^{0.357 \pm 0.020}$ $r = 0.993,\ n = 20$
Terrapene ornata
$y = 14.33\,x^{0.356 \pm 0.029}$ $r = 0.981,\ n = 26$
Trionyx spinifer
$y = 19.63\,x^{0.355 \pm 0.012}$ $r = 0.996,\ n = 30$

Pair-wise, inter-specific comparisons made on the logarithmic data resulted in $P < 0.05$ for certain comparisons. *Kinosternon flavescens* and *Chrysemys scripta* differed from one another; however, *T. ornata* did not differ from either of the former two species. *Trionyx spinifer* differed from all the other three species. Because significant differences were apparent for certain comparisons, no comprehensive equation was calculated.

DISCUSSION

Mosimann (1958) termed the b exponent a "growth coefficient" since it expresses an increase in one growth measurement with respect to another. The growth coefficients for the length-mass relationship of *T. ornata* and *K. flavescens* were similar to those of Meek (1982). The confidence intervals set about the exponent b in Meek's comprehensive equations included that of the two species examined here. The same conclusion held true for similar equations of other species in the literature (Mosimann, 1958; Dunson, 1967). This supports Meek's assertion that his comprehensive equation is representative of the growth relationship between length and mass of chelonians and that chelonians appear to increase these two parameters at equal rates.

Length-volume equations produced equivalent growth coefficients for the four chelonians. The growth coefficients presented by Mosimann (1958) were similar to those presented here. This indicates the concomitant increase of length and volume occurs at a similar rate in the chelonians examined.

The "a" value, a derivative of the y-intercept of the log-log regression, varied between species in the length-volume equations. The flat soft-shell turtle, *T. spinifer*, had a greater "a" value as opposed to the other, more domed species. Length shows a strong relative increase as opposed to other measurements in turtles (Mosimann, 1958). Dome shaped turtles (i.e., *T. ornata*, *K. flavescens*) exhibit a greater height : length ratio than does *T. spinifer*. The greater "a" value for *T spinifer* indicates this turtle must acquire a greater carapace length than the other species in order to achieve an equivalent volume. The low height : length ratio of the soft-shell results in a low profile body which facilitates rapid swimming.

Mosimann (1958) found growth coefficients to be consistent for length-mass and length-volume comparisons for a given chelonian species. He surmised that volume and mass increase at a similar rate for a species. The comparison of length-volume and length-mass growth coefficients indicates a slight disparity between the values for *K. flavescens* and *T. ornata*. However, length-volume growth coefficients for *C. scripta* and *T. spinifer* concur closely to length-mass growth coefficients given by Meek (1982) and Dunson (1967).

Meek (1982) stated allometric equations may be useful to compare growth relationships between species or populations. Seasonal changes in fatbody weight were evident through length-mass inter-sex comparisons in the desert iguana (Minnich, 1971). Length-volume equations appear to be quite applicable to describe other relationships. Inter-specific variation in reproductive capacity may be easier understood when clutch size is related to volume rather than carapacal length. Environmental adaptations, e.g., streamlined form, may also be inferred from morphometric data.

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POSSIBLE USE OF WATER TURNOVER RATES TO ESTIMATE FOOD INTAKE IN LIZARDS

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SUMMARY

The usefulness of using water turnover rates to estimate food intake in a small scincid lizard (*Lampropholis guichenoti*) was assessed in the laboratory. A significant relationship between food intake and water turnover measured using tritiated water was found, but this relationship has little predictive value as food intake accounted for only 35% of the variability of water turnover. Therefore, accurate estimates of food consumption cannot be made from water turnover values. We suggest that some of the lizards are compensating for increased preformed water intake with increased food consumption by reducing their drinking water intake, thus lowering their water turnover rates. This causes a large heterogeneity in the data, and this technique is thus probably only of general usefulness in field studies of non-drinking lizards.

INTRODUCTION

The quantitative assessment of food consumption by free-living animals has proved to be very difficult. To date, the easiest, most reliable method of directly measuring food consumption in free-ranging animals has been the use of ^{22}Na turnover rates (Green, 1978), and this technique has recently been validated and found useful for lizards (Gallagher, Morrison, Shine & Grigg, 1983). However, this technique relies on there being a significant correlation between Na turnover and food intake in the species under consideration. As well, the Na content of the diet of the species must be determined, and this is sometimes difficult to estimate in the field, particularly for herbivorous animals or where the diet changes seasonally (Gallagher *et al.*, 1983). Where this technique cannot be used, an easy, direct alternative is desirable.

A close positive correlation between water intake and food intake has long been recognized for a wide variety of animals (see Macfarlane & Howard, 1972), and water intake can thus be used to estimate food intake. Unfortunately, it is usually very difficult to accurately measure water intake in free-ranging animals, particularly if drinking water is available (Nagy & Costa, 1980). However, it has also been suggested that food intake correlates with water turnover (which only equals water intake if the animals are maintaining water balance) for several species of ruminants, including sheep (Morris, Howard & Macfarlane, 1962; Macfarlane & Howard, 1972; Benjamin, Degen, Breight, Chen & Tadmor, 1975) and cattle (Veroce, 1967). If this was also true for reptiles, it would be particularly useful in field studies of free-ranging free-drinking animals, as water turnover can be measured accurately using tritiated water (HTO) with little disturbance to the animals (Nagy & Costa, 1980).

In this paper, we present a laboratory assessment of the feasibility of using water turnover rates to estimate food intake in a small lizard, *Lampropholis guichenoti* (Lacertilia: Scincidae). This work is part of an investigation assessing radioisotope techniques for measuring food consumption and water turnover in these small Australian skinks.

MATERIALS AND METHODS

L. guichenoti is an insectivorous garden lizard common in south-eastern Australia, with an average body mass of 1 g and a snout-vent length of 4 cm. Specimens are kept easily in captivity, and were obtained in Sydney, chiefly from private gardens.

Twelve animals were kept in separate plastic containers 30 × 20 × 15 cm in a laboratory maintained at a temperature of 25°C and a humidity of 50%. These lizards are active under these conditions in the field in Sydney, and the experimental animals were freely active in the laboratory. They were supplied with drinking water *ad libitum*, both during and for 4 weeks prior to the experiment. Four groups of three lizards each were fed rates of 1, 2, 3, or 4 *Dacus tryoni* (Queensland fruitfly) pupae per day. All pupae were of equivalent mass, and all food presented was consumed.

The use of HTO to measure water turnover in small lizards requires modifications to be made to the usual methods, and the assumptions inherent in the techniques described below are discussed by Gallagher & Taplin (1985). The lizards were labelled with a single intraperitoneal injection of 2.5 μl of HTO with a specific activity of 2.0 mCi/ml (74 MBq/ml). The tritium content of the urine was then determined 3 hr after injection, and twice daily for the next 8 days. The urine samples were collected into a microcapillary tube held against the cloaca, counted for 5 min in a Packard Tri-carb Liquid Scintillation Spectrometer Model 3375, and the counts were corrected for background and quenching. The fractional rate of water turnover (k) was then calculated from the slope of the least squares linear regression of ln HTO activity versus time for each lizard. Total body water (TBW) was measured by isotope dilution, with the initial tritium activity in the urine being estimated for each lizard by calculating the y-intercept of the regression of ln HTO activity *vs.* time. The rate of water turnover (WT) in ml/day was then calculated for each lizard from:

$$WT = \mathrm{TBW} \times k$$

This equation assumes that TBW remains constant throughout the measurement period, and so each animal was weighed daily to detect possible changes in the size of the water pool.

RESULTS

The water turnover rates (Table I) were corrected for the 6.2% overestimation of total body water found for *L. guichenoti* by Gallagher & Taplin (1985) using the isotope dilution technique. None of the lizards showed a change in body mass greater than 1% during the experiment, and so they were assumed to be in steady state with respect to water.

The relationship between water turnover corrected for mass-specific water requirements (Gallagher & Taplin, 1985) and food intake was estimated by least squares linear regression (Fig. 1). An analysis of

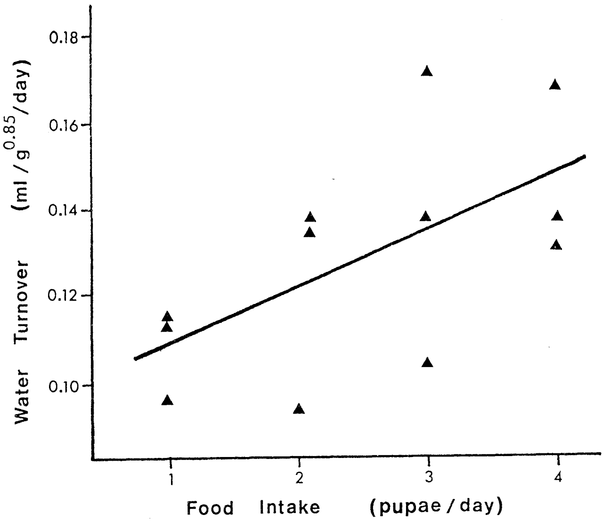

FIG. 1. Relationship between mass-corrected water turnover and food intake in 12 *L. guichenoti*. Line fitted by least squares linear regression is $y = 0.013x + 0.010$.

TABLE I. Comparison of water turnover rates in *L. guichenoti* measured under varying feeding regimes

Food intake (pupae/day)	Lizard mass (g)	Corrected total body water (%)	Water turnover (ml/day)	Deviation of mass-corrected water turnover from predicted turnover (%)
1	0.93	73.5	0.108	−12
	1.17	72.8	0.129	+4
	1.59	78.3	0.142	+6
2	1.19	78.1	0.108	−26
	0.93	72.0	0.126	+10
	1.08	71.9	0.147	+13
3	1.53	74.7	0.150	−23
	1.24	73.0	0.166	+2
	1.34	76.2	0.221	+27
4	0.93	70.8	0.123	−11
	0.99	68.7	0.137	−7
	1.37	75.9	0.220	+14

variance ($F = 5.47$, d.f. = 1, 10, $0.01 < P < 0.05$) indicates that the slope of the regression line is significant, but the regression line accounts for only 35% ($r^2 = 0.35$) of the variability of the dependent variable (water turnover).

These results suggest that, while water turnover could be used to estimate food intake in *L. guichenoti*, it would be of little practical value. The regression would need to be significant at $P < 0.01$, with $r^2 > 0.80$, in order to be able to predict food intake from water turnover values with any accuracy (*c.f.* Gallagher *et al.*, 1983).

DISCUSSION

While there is an obvious trend in the data towards increased water turnover with increased food consumption, this trend is of little predictive value for this species, as there are large deviations from the regression line (Table I). Water turnover also correlates with many other variables, which could interact to create the large heterogeneity apparent in the data.

Probably the most important factor causing the discrepancy between water turnover and food intake is the amount of free drinking water available to the lizards. Animals gain water from free drinking water, preformed water in food, and the metabolic oxidation of foodstuffs. However, the metabolic water input is usually low (Minnich, 1982), and will be constant under steady-state conditions. There will thus be a strict coupling of water and food intake in non-drinking animals. On the other hand, if free drinking water intake is high relative to water intake from food, then this relationship may be obscured by high rates of water turnover if the increased intake is excreted rapidly.

Since the lizards in our experiment were assumed to be in steady state with respect to water, water turnover will equal water intake, and an assessment of drinking water consumption can be made. The pupae used as a food source had an average water content of 8.8 μl (SE = 0.1, $n = 10$) each, and thus were contributing an average of 7%, 14%, 15%, and 23% of the total water intake for the lizards fed 1, 2, 3, and 4 pupae/day respectively. The only reports of metabolic water input for insectivorous lizards are 19% (for *Uma notata;* Deavers, 1972), and 39% (for *Uma scoparia;* Minnich & Shoemaker, 1972) of the total water intake. These values are for non-drinking desert lizards, and the percentage would be even lower for lizards such as *L. guichenoti* from more mesic habitats (Minnich, 1982). Thus, drinking water probably contributes 50–70% of the total water intake of *L. guichenoti*, and even small variations in this amount would significantly alter the rate of water turnover independently of food intake. We could not directly measure the actual drinking water intake of these lizards, as the daily volumes involved were too low to be determined accurately (see Gallagher & Taplin, 1985).

That drinking water contributes less to the total water intake of *L. guichenoti* as the dietary water input increases is to be expected. As none of the lizards showed a change in body mass, they were not storing water. Therefore, when dietary water input increases, some of the lizards are regulating their body water volume by reducing their drinking water intake (which reduces overall water turnover), rather than by increasing their water output (which would increase turnover). The extent to which lizards use this form of compensation varies between individuals, and this would account for some of the variability in the water turnover rates within each feeding treatment (Fig. 1).

It thus appears that water turnover does not provide a reliable means of estimating food intake in active, free-drinking *L. guichenoti*, and would therefore be of little value in studies of free-ranging populations if drinking water was available during the study. The availability of drinking water in the field is usually erratic (pers. obs.), and consequently the use of water compensation mechanisms would be even more variable among individuals in the field than among laboratory animals. Little is known about water intake rates in reptiles which have access to drinking water in the field (Minnich, 1982; Nagy, 1982), but it is likely that this compensation mechanism is common. This suggests that the use of water turnover rates may only be of general applicability among non-drinking reptiles, and this technique has been used in a limited way for several non-drinking desert lizards in the field (*e.g.* Minnich & Shoemaker, 1970; Nagy & Shoemaker, 1975; Congdon, Vitt, Van Loben Sels & Ohmart, 1982).

Therefore, in situations where the use of ^{22}Na is impossible or complex, recourse should be made to more tedious methods of directly estimating food consumption, such as establishing a relationship between faecal production and food intake (*e.g.* Avery, 1971, 1978, 1981; Andrews & Asato, 1977).

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MORPHOMETRIC OBSERVATIONS OF SMOOTH AND PALMATE NEWTS IN MID-WALES

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SUMMARY

During a three-year study, the sizes of newts breeding at Llysdinam pond were shown to be fairly constant. Average lengths were: male palmate newt (excluding tail filament), 66.8 mm: female palmate newt, 76.5 mm: male smooth newt, 82.1 mm and female smooth newt 79.8 mm. Frequency distributions of total length are statistically normal. Male smooth newts have a proportionally longer tail than male palmate newts, and this could be related to differences in courtship display.

INTRODUCTION

Since the work of Boulenger (1894) and Evans (1984). detailed studies on the size of the three species of newt present in Britain (the smooth newt *Triturus vulgaris*, the palmate newt *T. helveticus* and the crested newt *T. cristatus*) have been relatively scarce, although some data are available for continental populations (van Gelder 1973). In his autecological studies of smooth newt populations in Oxfordshire, Bell (1977) analysed size data in some detail, but apart from Bell's earlier work (1966, 1970) on newts in Leicestershire, there have been few studies on size in newt populations from other localities. Reference to size has been included in some general accounts (Steward, 1969; Smith, 1973; Arnold & Burton, 1978) but in many cases sample sizes are small and statistical treatment is absent so the information is of limited use for comparative purposes. The following account presents data on length and body proportions for smooth and palmate newts, collected over a three year period from a breeding site in mid-Wales, with supplementary data from other localities. Considerable numbers of animals were handled, allowing differences in size and body proportions between species, sex and breeding season to be investigated.

METHODS

Samples of smooth and palmate newts were collected during their 1981, 1982 and 1983 spring migrations to Llysdinam Pond, Newbridge-on-Wye (grid reference SO 009586). The pond was completely encircled by a polythene barrier and pitfall trap system, which proved to be highly effective at trapping large numbers of newts (Harrison, Gittins & Slater, 1983). The pitfall traps were inspected daily and any newts captured moving into the pond were taken to the laboratory to be measured. Individuals were placed on graph paper and total length (snout to tip of tail) and body length (snout to posterior of cloacal swelling) were measured to the nearest 1 mm. The length of the tail filament in male palmate newts was excluded as this is a sexual feature which undergoes seasonal changes in length (Smith, 1973). After measuring, newts were released on the opposite side of the barrier. Newts were also collected from a number of other breeding sites in mid-Wales, by direct netting or trapping, and were processed in an identical manner to the Llysdinam material.

Statistical analyses were performed using the Minitab (Ryan, Joiner & Ryan, 1981) and Microstat (Microsoft Ltd.) statistical packages.

RESULTS

With the exception of male smooth newts, which were longer in 1981 than in the other two seasons, there were no significant differences in length between years for any of the other sex/species categories (Table I). The overall average lengths of newts breeding at Llysdinam were not significantly different from those collected from other mid-Wales localities (Table I), suggesting that, in terms of the sizes of individuals, the Llysdinam populations are typical of the area.

Frequency distributions of total length show no distinct signs of polymodality and are statistically normal, the only exception being the sample of female palmate newts in 1982 (Fig. 1).

In all samples the majority of individuals had tails shorter than the combined length of head and body. The ratios of tail length : body length were similar for females of both species but differed in males—male smooth newts having a relatively longer tail and male palmate newts a relatively shorter tail than females (Table II). Although the values of the ratios differ somewhat, similar patterns were observed in the samples of newts from other localities (Table II).

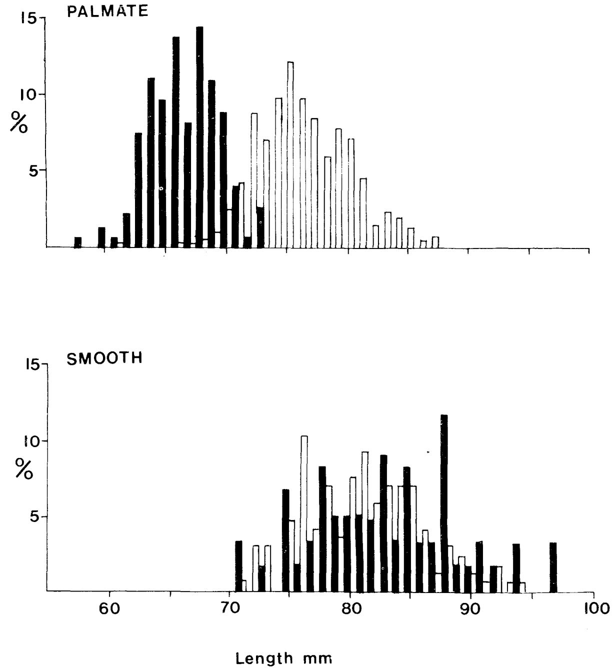

FIG. 1. Total length frequency distributions for smooth and palmate newts (Llysdinam 1981 material). Males–filled columns; females–unfilled columns. Shapiro-Wilk tests indicate that distributions are statistically normal:

Male palmate: $r = 0.998$, $n = 142$, $P < 0.01$
Female palmate: $r = 0.991$, $n = 388$, $P < 0.01$
Male smooth: $r = 0.999$, $n = 62$, $P < 0.01$
Female smooth: $r = 0.996$, $n = 175$, $P < 0.01$

Total length frequency distributions for 1982 and 1983 are statistically normal ($P < 0.01$), the only exception being the sample of female palmate newts in 1982.

TABLE I. Mean lengths (mm) of newts breeding at Llysdinam and at other mid-Wales localities. Male smooth newts at Llysdinam, 1981, are significantly larger than in 1982 ($t = 2.37$, $P < 0.05$) and 1983 ($t = 2.49$, $P < 0.05$). All other annual differences are not significant ($P > 0.05$). Differences between Llysdinam overall lengths and those found in other localities are not significant ($P > 0.05$).

		Male Palmate			Female Palmate			Male Smooth			Female Smooth	
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD
Llysdinam 1981	142	66.7	2.9	388	76.2	4.0	62	83.6	5.9	175	80.1	5.4
Llysdinam 1982	30	66.7	2.9	30	76.9	4.1	30	80.6	5.6	30	78.9	5.9
Llysdinam 1983	45	67.2	3.5	66	76.5	4.1	33	80.9	4.5	21	78.7	5.9
Llysdinam overall	217	66.8	3.0	200	76.5	4.1	125	82.1	5.7	266	79.8	5.2
Other mid-Wales localities	13	68.1	5.1	27	78.1	5.5	23	83.9	5.4	13	82.2	5.4

TABLE II. Median values of tail:body ratios for the Llysdinam newt populations and those at other mid-Wales localities. Kruskal-Wallis one way analysis of variance shows year to year variation to be significant only in the case of male smooth newts ($H = 14.7$). Mann-Whitney tests show no significant difference between overall tail:body ratio of Llysdinam female palmate and female smooth newts ($Z = -0.81$, $P > 0.05$). Overall tail: body ratio for male palmate newts is significantly smaller than for females ($Z = 4.35$, $P < 0.01$). Overall tail:body ratio for male smooth newts is significantly larger than females ($Z = 6.52$, $P < 0.01$).

	Male Palmate	Female Palmate	Male Smooth	Female Smooth
Llysdinam 1981	0.77 ($n = 30$)	0.82 ($n = 30$)	0.94 ($n = 30$)	0.84 ($n = 30$)
Llysdinam 1982	0.79 ($n = 30$)	0.80 ($n = 30$)	0.91 ($n = 30$)	0.83 ($n = 30$)
Llysdinam 1983	0.79 ($n = 30$)	0.83 ($n = 30$)	0.88 ($n = 30$)	0.83 ($n = 21$)
Llysdinam overall	0.78 ($n = 90$)	0.82 ($n = 90$)	0.91 ($n = 90$)	0.83 ($n = 81$)
Other mid-Wales localities	0.79 ($n = 13$)	0.87 ($n = 27$)	0.92 ($n = 23$)	0.91 ($n = 12$)

DISCUSSION

The use of total length as a measure of size in newts must be viewed with some caution. Newts may lose their tails to predators and subsequently have to regrow them. If this were a frequent occurrence, it would lead to a large variation in the lengths of individuals in a population and make statements about their average size meaningless. However, the relatively small standard deviations of total length found for the Llysdinam populations (Table I) indicate that individual variation in length is small and that newts which are unusually small as a result of tail damage may be uncommon in these populations.

The average sizes of newts breeding at Llysdinam remained fairly constant over the three-year-study period. Somewhat different findings are described by van Gelder (1973) who found the average sizes in a Netherlands population of palmate newts to vary considerably from year to year. Despite this annual variation, however, the palmate newts studied by van Gelder were much smaller than those described here, with overall mean lengths of 59.6 mm for males (excluding filament) and 64.2 mm for females. Conversely, Evans (1984) found the sizes in a Scottish population of palmate newts to be larger than those in mid-Wales, with average sizes of 75.2 mm (males, including filament) and 79.7 mm (females). Bell (1966) found the sizes of palmate newts in Leicestershire to be similar to the mid-Wales populations but recorded smaller average lengths for smooth newts (males 78.9 mm, females 75.9 mm). Such geographical variation may be related to geographical factors, such as climate or habitat suitability, but more data based on large samples from a variety of habitats are needed to investigate this further.

Hagstrom (1977) used bone sectioning methods to study ages and growth rates of newts and found that, compared to the growth during the first two years of life, adult growth seems to add very little to the animals size. Similarly, in studies of the post metamorphic growth of the crested newt, Glandt (1981), found that growth slows after sexual maturity, with some individuals not growing at all or even exhibiting negative growth. Since juvenile newts are thought not to migrate to breeding sites (Steward, 1969) it is likely that the data presented here refer to adult newts, and the absence of distinct age classes in the length frequency distributions are, therefore, consistent with the findings of Hagstrom and Glandt. By measuring body length to an accuracy of 0.1 mm, Bell (1977) was able to investigate smooth newt population age structures by interpreting length frequency distributions. Measurement to such fine limits possibly allows the very slight growth which occurs during adult life to become apparent in the length frequency distributions, although Hagstrom questioned the use of size for estimating the age of adult newts and Glandt states that "population ecologists are not able to get reliable information about age structure of a population from length frequency data".

The vast majority of newts in this study had tails which were shorter than the combined length of the head and body. This is in agreement with the observations of Bell (1966) but is the reverse of the data given by Smith (1973) and the other studies quoted there. It is likely that much of the discrepancy may result from non-standard measuring techniques, for example, taking body length from snout to the centre of the cloaca rather than the hind margin would increase the length of tail relative to body, and the inclusion of the tail filament in male palmates would have the same effect.

The relationship between body proportions and the presence of webbing of the hind feet in a number of European newts (*T. vulgaris*, *T. helveticus*, *T. cristatus* and *T. alpestris*) has been discussed by Beebee (1980). Comparing the ratios of body volume:tail area, he found that male smooth and male palmate newts have larger tails than conspecific females and both sexes of the other species. Consequently, he suggests, foot webbing in male smooth and palmate newts has evolved to counteract the potential imbalance caused by the relatively bulky tail whilst descending from the water surface. However, using the simpler ratio of tail length:body length on relatively large sample sizes, the present study suggests that there may be further differences between the relative sizes of the tail in male smooth and male palmate newts. Halliday (1975) has described various morphological differences between males of these species, such as the extent of development of the dorsal crest and the dorso-lateral ridge, and related these to differences in courtship activities. Here we suggest that differences in relative tail size between male smooth and palmate newts may also be related to courtship display. A detailed comparison of courtship in smooth and palmate newts is given by Halliday (1975, 1977) on which the following account is based. Each display consists essentially of the same three basic actions: wave, whip and fan. The whip action features prominently in the male smooth newt's display. This is a violent action in which the whole length of the tail is lashed against the side of the body. It seems reasonable to assume, therefore, that a large tail is best suited to this activity. In the display of the male palmate newt, fan features more prominently: this is a more delicate action consisting of high frequency vibrations of the tail, and a smaller lower mass tail is possibly more appropriate to this activity.

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THE RESPONSES OF INSEMINATED FEMALE SMOOTH NEWTS, *TRITURUS VULGARIS*, TO FURTHER EXPOSURE TO MALES

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SUMMARY

Female smooth newts are sometimes multiply inseminated as a consequence of competitive interactions between males (sexual interference). After insemination, most females remain unresponsive to further exposure to males for up to 20 days. However, some will remate during this time, and such behaviour may result in sperm competition.

INTRODUCTION

In many species of urodele amphibians, especially those that breed in water, there is a considerable potential for females to engage in matings with more than one male. Indirect evidence for this is provided by the observations that adult sex ratios at breeding sites are often skewed towards an excess of males, males commonly produce vast numbers of spermatophores and, within single sexual encounters at least, females may accept more spermatophores than are probably required to fertilize their ova (reviewed by Halliday & Verrell, 1984). Direct evidence for multiple mating by female urodeles is of two types. Biochemical and genetical studies have revealed multiple paternity in clutches of the progeny of the European alpine newt, *Triturus alpestris* (Rafinsky, 1981), and the North American dusky salamander, *Desmognathus ochrophaeus* (Labanick, 1983; Tilley & Hausman, 1976). Direct behavioural evidence for the insemination of females by more than one male is available for the European smooth newt, *Triturus vulgaris*. In this species, males mimic behaviour patterns normally shown by females and will interfere with one anothers' attempts to court and inseminate females. In two out of 45 such competitive encounters (4.4%), females picked up sperm from two males (Verrell, in press).

Nothing is known about the sexual responsiveness of inseminated female smooth newts, although this issue is clearly important in a consideration of multiple insemination. In this paper, an experiment is described which was conducted to determine the extent to which female *T. vulgaris* engage in multiple matings.

MATERIALS AND METHODS

Smooth newts were collected in the spring of 1981 as they migrated to ponds in the Oxford and Milton Keynes areas of southern England. These animals had thus not yet mated in that year. Twenty five females were each paired with a male in an aquarium measuring 61 × 30 × 30 cm and permitted to pick up a single spermatophore (the "initial insemination"). Each was then housed individually in a plastic trough containing aged tap water at about 18°C and a few fragments of water weeds. Food was supplied ad libitum, and consisted of chopped-up earthworms. In order to test female responsiveness, each female was subsequently placed with a sexually active male at fixed intervals of 1 h, 12 h and 2, 4, 8, 16 and 20 days after the initial insemination. Each test lasted for a period of 30 min maximum, or for a single courtship encounter. Males used were selected from a stock supply, and were considered sexually active if they responded to a female held in a strait-jacket with courtship behaviour (see Halliday, 1976).

The responses of the females during each test were classified according to the following qualitative scale:

(1) No response: the females responded to male approach by consistently fleeing.

(2) Weak response: the females responded to the initial stages of male courtship by approaching or remaining close to the males.

(3) Females elicited the deposition of at least one spermatophore, but did not pick it up.

(4) Females elicited the deposition of at least one spermatophore, of which at least one was picked up.

RESULTS

The responses of 25 inseminated female *T. vulgaris* to further exposure to males are summarized in Table I. The majority of females (72%) were relatively unresponsive to further exposure to males over a period of 20 days post initial insemination; all were exposed to at least the static display phase of courtship (see Halliday, 1976).

TABLE I. The responses of 25 inseminated female smooth newts to further male courtship. Not every female was tested at each time interval (see text for further explanation).

Type of response	Number of females	Time since initial insemination/hours
No response	12	All up to 480
Weak response	6	1, 1, 1, 12, 12, 48
Spermatophore deposition only	5	12, 12, 192, 384, 480
Spermatophore pick up	2	12, 48

Only two (8%) of the females were inseminated for a second time in 20 days; a further five females (20%) followed male courtship through to the spermatophore deposition phase, but did not pick up sperm. Adding these figures together to derive a score for the "potential" number of females multiply inseminated due to remating gives 28% of all females tested.

Small sample sizes prevent a detailed consideration of the time course of changes in female responsiveness after initial insemination. However, the data indicate that females which remained sufficiently responsive to elicit spermatophore deposition, with or without pick up, did so either soon (12–48 h) or a long time (192–480 h) after initial insemination.

DISCUSSION

The results presented above demonstrate that, after insemination, most female *T. vulgaris* become unresponsive to further male courtship and remain so for as long as 20 days. Of those that remained at least weakly responsive ($n = 13$), approximately three quarters became unresponsive after 48 hr.

In many species, females become either unresponsive to further courtship or unattractive to males soon after insemination. In the lizard *Anolis carolinensis*, the act of mating literally "switches the female off" (Crews, 1973), whereas in the garter snake *Thamnophis radix*, the female is rendered unattractive by odours present in the male's copulatory plug (Ross & Crews, 1978). It is not clear what type of mechanism is operating in female smooth newts, but it does seem that they remain sufficiently attractive after insemination to elicit courtship from males. Perhaps the neuroendocrine events which probably follow insemination and ultimately result in ovulation are responsible for the decline in female responsiveness (see Moore, McCormack & Swanson, 1979).

Only two females picked up sperm from two males in the present study, although the "potential" for multiple insemination was as high as 28%. These data, together with those concerning multiple insemination due to sexual interference (Verrell, in press), raise the possibility that some females may build up a stock of sperm to which several males contribute. Female newts store viable sperm in a specialized cloacal structure known as the spermatheca (reviewed in Halliday & Verrell, 1984), and it is possible that sperm competition (*sensu* Parker, 1970) occurs in some females. The outcome of such competition regarding paternity is hard to predict; if the ejaculates from different males remain discrete in the female's genital tract, an advantage may accrue to either the first or last male to mate, but if they mix, paternity may be equally shared (Parker, 1970).

Whether females which remate gain any advantage from so doing is unknown. It may enable them to increase the genetic heterogeneity of their offspring and/or act as an "insurance" against mating with subfertile males (Walker, 1980). In the present study, 17 females began laying eggs between 2 and 6 days after initial insemination, and their subsequent sexual responsiveness bore no clear relationship to such laying; it seems unlikely that they remained responsive in order to replenish exhausted sperm supplies. In some insects, females acquire nutrients as well as sperm during mating, and multiple mating may be as much a nutritional strategy as a sexual one (Wiklund, 1982). Urodele spermatophores contain a variety of substrates which may be of use to females nutritionally, such as mucopolysaccharides (Russell, Brandon, Zalisko & Martan, 1981). The intriguing possibility that female newts actually utilize such materials has yet to be investigated.

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PHYSICAL ABNORMALITIES OF THE LIMBS OF SMOOTH NEWTS (*TRITURUS VULGARIS*) (SHORT NOTE)

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Several types of abnormal regeneration in urodele limbs have been observed in the laboratory following amputation (Schwidefsky, 1935; Scadding, 1981), limb transplant induction (Newth, 1958) or chemical treatment (Breedis, 1952). Griffiths (1981) described some similar, naturally occurring abnormalities in a smooth newt (*Triturus vulgaris*) population in London. He noted three kinds of abnormality, (i) supernumary or bifurcate toes, (ii) loss or atrophy of toes and (iii) accessory limb growth. A fourth type of abnormality, webbing between the toes, can occur in the atypical regeneration of laboratory amputated fore and hind limbs of the smooth newt, the crested newt (*T. cristatus*) and the alpine newt (*T. alpestris*; Schwidefsky, 1935) and it has also been observed in a population of *T. boscai* in Portugal (Malkamus, 1981). However, Griffiths did not report this abnormality in his population.

Smooth newt males can be easily recognized during the breeding season when they possess a red-blue tail stripe, an undulating crest that is continuous with the tail and a spotted body and throat. The hind toes of the smooth newts in good breeding condition are fringed with delicate lobes of skin while those of the male palmate newt (*T. helveticus*) exhibit proximal webbing (Halliday, 1975; Halliday & Joly, 1980). On 4 April 1983, an adult smooth newt was found on a routine collecting visit to a garden pond in Oxford, with proximal webbing on one hind limb (Fig. 1). The toes of the right limb were fully webbed and they were up to 2.0 mm, mean 1.05 mm (16.5%) shorter, when measured from the ventral foot spur to the tip of the toe, than the corresponding toes on the left limb (Male A, Table I); these appeared to be normal in all respects. Apart from the typical male smooth newt colour and patterning (light brown with dark spots), the

Fig. 1. Male smooth newt with abnormally webbed hind limb.

webbed limb bore a remarkable resemblance to the palmated hind limb of a *T. helveticus* male in breeding condition. Twenty-six of the animal's physical measurements (e.g. snout-vent length, girth, etc.) were recorded as part of a separate morphometric study of the smooth newt population in the pond. In addition, the male's courtship display was observed in the laboratory and compared to Halliday's (1975) description of smooth newt courtship. These data (J. M. Roberts, unpublished) indicated that the animal's morphological characters and general courtship behaviour fell within the normal range for the smooth newt.

Other, apparently normal smooth newt males with one fully webbed hind limb have subsequently been found in 1983 in two different populations. One male was discovered in a farm pond at Milton Bryan, Bedforshire (left foot webbed) and two males were netted in a woodland pond at Farnham, Buckinghamshire (one right foot webbed, one left foot webbed). No data were available for the former male but, similar to male A, the latter two animals had generally shorter digits (Males B and C, Table I) on their webbed limb than on their normal limb. The digits of Males B and C were up to 1.75 mm, mean 1.20 mm (19.3%) shorter, and 1.75 mm, mean 1.20 mm (12.5%) shorter, respectively.

The *T. vulgaris* population from Farnham co-existed with populations of *T. helveticus* and *T. cristatus*. The possibility that the abnormal webbing found on these animals was due to either hybridization between smooth and palmate newts, or was a product of gross chromosome change, was investigated by examining spermatogonial metaphase bivalents from one animal. The chromosomes were C-banded using a modification of Sumner's (1972) barium hydroxide/hot saline method. The chromosomes had paired normally and the C-banding patterns compared well with those presented by Schmid *et al.* (1979) for *T. vulgaris*. Thus there was no evidence for either the presence of hybrid chromosomes or gross chromosome abnormalities. Hybrids of *T. vulgaris meridionalis* and *T. helveticus helveticus* tend to exhibit intermediate morphology (Spurway & Callan 1960) and so the normal physical characters and chromosomes of the Farnham male both suggest that atypical webbing is not a result of hybridization.

Additional information about partial webbing (between two or more toes) and other limb abnormalities has been obtained from a farm pond in Soulbury, Buckinghamshire. The pond was surrounded by a drift fence and adult newts were intercepted as they migrated to the pond to breed in the spring of 1983. Also, newts were collected from the water by dip-netting and underwater trapping. A total of 636 animals (233 males, 403 females) were examined and 29 of these (4.56%) possessed at least one type of limb abnormality. Details of these are presented in Table II. These data are too sparse for a quantitative analysis: nevertheless, it seems that males (4.7%) and females (4.5%) exhibit limb abnormalities to the same extent. Some comparisons with the data of Griffiths (1981) are possible. His population of 268 newts had 79% more supernumary or bifurcate toes, 25% more lost or atrophied toes and 60% more accessory limbs than did the newts in the Soulbury population. Griffiths also observed a greater incidence of abnormalities in the hindlimbs compared to the forelimbs—the data presented in Table II support this, as 65.5% of the limb abnormalities were found in the hindlimbs.

TABLE I. Difference in millimetres in length of corresponding webbed and normal toes when measured from the ventral foot spur to the tip of the toe. Toe 1 = nearest to cloaca, Toe 5 = furthest away from cloaca.

	Hind toe number				
Male	1	2	3	4	5
A	0.25	0.50	2.00	1.25	1.25
B	0.75	1.50	1.75	1.25	0.75
C	0.00	0.50	0.75	1.75	0.50

TABLE II. Data on the occurrence of limb abnormalities in a population of 636 Smooth Newts at Soulbury, Buckinghamshire.

	Number of animals			
	Forelimbs		Hindlimbs	
Abnormality	Male	Female	Male	Female
Supernumary/ bifurcate toes	0	2	3	0
Loss/atrophy of toes	2	6	2	8
Webbing between toes	0	0	2	2
Accessory limbs	0	0	2	0

The cause of abnormal limb growth is unclear. Griffiths suggests that abnormal regeneration, following injury, may be a major cause and empirical support for this view is provided by the work of Scadding (1981) on regeneration in salamanders. Various types of tissue injury, which could lead to regeneration, have been observed in the Soulbury population, the most severe consisting of the complete absence of one limb in two males. Hence it is possible that the unusual webbing and shorter digits of males A, B and C from Oxford and Farnham represent intermediate stages in the progressive abnormal regrowth of a lost limb. Abnormalities may also arise spontaneously, without injury or regeneration, due to disturbed development. For example, Cooke (1981) has found that some agrochemicals can induce skeletal abnormalities in anuran tadpoles. Thus, the higher proportions of limb abnormalities found in Griffith's population in London may be due to greater levels of damage caused by heavier pollution or predation. There are several possibilities, but causation of limb abnormalities in newts clearly requires further empirical investigation.

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