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HABITAT AND OTHER ENVIRONMENTAL CHARACTERISTICS OF THE NATTERJACK TOAD (*BUFO CALAMITA* LAUR.) IN SWEDEN

CLAES ANDRÉN AND GÖRAN NILSON

University of Göteborg, Department of Zoology, Box 250 59, S-400 31 *Göteborg, Sweden*

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SUMMARY

Ground and vegetation structure breeding pool characteristics, climate, competition, predation, and negative effects of human activities in natterjack toad (*Bufo calamita*) populations along the Swedish west coast were compared with those in coastal and inland populations in the southernmost parts of the country. The west coast localities were almost free from shading vegetation with bare and smooth ground, while the southern localities were more forested and grass covered and the bare ground mainly consisted of sand. The number of breeding pools were generally higher in the west coast populations, and they were smaller in size, less deep, had lower shore vegetation, and were almost exclusively situated on rocky ground.

Precipitation and mean air temperature were lower in spring but higher in autumn in the west coast localities, and the number of sunshine hours were higher throughout the active season at the west coast compared with south Sweden. Competition, predation and negative effects from human activities may be higher in the south Swedish populations than in the west coast natterjack islands.

INTRODUCTION

The natterjack toad has a wide range in Europe from western U.S.S.R. through central Europe to France and Spain in the south. The northern limit extends from Ireland and south Scotland through Denmark and south Sweden to Estonia. Throughout its range the natterjack seems to prefer dry sandy or stony soil, and the preference for sandy soils and open unshaded terrain may be most stringent around the northern border (Beebee, 1979).

The Swedish range is divided into two separate areas. One includes the southern counties Skåne and Blekinge (and one uncertain locality in southernmost Halland), and the other extends over the southern and central parts of the county Bohuslän along the west coast (Fig. 1). The distribution in Sweden has gradually decreased and this is most obvious in the southern inland localities (Berglund, 1976; Ahlén & Berglund, 1980). For this reason the geographical separation between the two areas has been most pronounced during the last century.

The natterjack populations along the Swedish west coast are restricted to small and rocky islands in the outermost archipelago (Andrén & Nilson, 1979, 1983, 1984). We do not know how long these islands have been colonized by the natterjacks or how dispersal to and between islands has been possible as the salinity of the surrounding seawater (about 2.8%) is lethal to both tadpoles and adults. Ecological conditions on the west coast islands differ from those in other parts of the species range, and the west coast natterjacks are significantly different in some morphological characters compared with both south Swedish and Continental populations (Nilson & Andrén, in prep.).

FIG. 1. The natterjack toad (*Bufo calamita*) distribution in Sweden. Open circles are observations made before 1959 (From Gislén & Kauri (1959)). Filled circles are own observations after 1959. Figures are counties (1) Bohuslän, (2) Halland, (3) Skåne and (4) Blekinge.

In this study we compare the habitat, breeding pool characteristics, climate, competition, predation, and negative effects of human activities in the west coast populations, where vegetation, climate and chemico-physical conditions of breeding pools are marked by the maritime influence, with that of south Swedish coastal and inland natterjack populations living in more "traditional" sandy areas.

STUDY AREAS AND METHODS

The study was performed during May, June and July in 1980 in three different areas in Sweden, (1) on small islands off the west coast, (2) along the southernmost coast and (3) in an inland area in southernmost Sweden (see Fig. 1). In each area five more or less isolated localities were selected, all of which had comparatively dense and stable natterjack populations. In Table I latitudes, altitudes and distances to the sea are given for all localities included in the study.

The area used by the natterjacks was determined for each locality and the macrohabitat (woodland, bush, grass, bare ground) and ground structure (earth, sand, gravel, solid rock) were estimated as a percentage of total activity area. We counted the number of breeding pools used, and their mean area, mean maximum water depth, free water surface, and shore vegetation height were noted. For each pool the shore line substrate (earth, sand, gravel, rock, vegetation) and shore line inclination were determined.

The climatic variables which were believed to affect tadpole development and survival (monthly rainfall, mean air temperature and number of daily sunshine hours) were collected from the nearest meteorological station. (For the west coast populations this was a station situated on one of the natterjack islands.) The values given are means for 30 years (1931–1960) or 15 years (1961–1975, for sunshine hours). Potential competitors and predators on both larval and adult stages observed or known to occur within the locality were noted, but the effects of competition or direct predation were not observed during the study. The character of the surrounding land (grazed, arable, industrial, recreational) was noted for all localities studied.

RESULTS

Habitat

The distribution of macrohabitat and ground structure in all three areas investigated are given in Table II. The west coast island localities were almost free from shading vegetation with bare and smooth hilly ground and with grass or small bushes limited to crevices and cracks. The bare ground almost exclusively consisted of solid rock (Fig. 2). The localities along the southernmost coast and in the south inland did not differ among themselves or between the two areas in the structure of cover of vegetation or in the ground structure. In comparison with the west coast natterjack islands the southern localities were generally more forested, the grass covered areas were larger and the proportion of bare ground was more limited and mainly consisted of sand (Fig. 3).

Table I. The latitudes, altitudes and distances to the sea for all natterjack toad (*Bufo calamita*) localities included in this study.

	West coast localities ($n = 5$)	South coast localities ($n = 5$)	South inland localities ($n = 5$)
Latitude (°N)	57.54–58.06	55.24–56.33	55.32–55.41
Altitude (m)	0	0–15	20–40
Distance to sea (km)	0	0–5	10–27

Table II. Macrohabitat and ground structure in natterjack toad (*Bufo calamita*) localities in Sweden

	West coast locality ($n = 5$) Mean ± SD	South coast locality ($n = 5$) Mean ± SD	South inland locality ($n = 5$) Mean ± SD
Macrohabitat (%)			
Woodland	<1	8 ± 13	23 ± 19
Bush	<1	5 ± 4	12 ± 4
Grass	3 ± 2	61 ± 35	21 ± 15
Bare ground	97 ± 2	26 ± 36	35 ± 36
Ground structure (%):			
Earth	3 ± 2	26 ± 22	23 ± 18
Sand	<1	71 ± 20	67 ± 26
Gravel	<1	1 ± 2	10 ± 17
Solid rock	97 ± 2	2 ± 5	< 1

Breeding Pools

The information on breeding pool characteristics for all localities is summarized in Table III. The number of breeding pools was generally higher in the west coast populations than in the southern localities, but variation was high in both areas. The mean water surface area was smaller in the west coast breeding pools, but the variation in the southern localities was very high (from 2 to about 10 000 m^2 in temporary flooded areas). In one south coastal population breeding occurred in a bay of the Baltic Sea with a salinity of about 0.4%. This is not possible along the west coast where the salinity is about 2.8%. Mean maximum water depth in the west and south coastal populations was generally lower than in the inland breeding pools, but egg deposition normally took place close to the shore or at least at low water depth (10–15 cm) in all breeding pools.

The proportion of free water surface in the pools was similar in all three areas studied. The height of shore vegetation was low in the west coast pools and higher and more varying in height in the southern coastal and inland breeding pools. Shore line substrate was mainly bare rock interrupted by grass vegetation in the island natterjack breeding pools at the west coast, but more varying and less dominated by rock in the southern localities. The inclination of breeding pool shores was similar in all three areas investigated, and on average at least 50% of the pond shore line was flat (inclination less than 10°).

FIG. 2. A typical natterjack toad (*Bufo calamita*) habitat from the outer archipelago off the Swedish west coast.

TABLE III. Breeding pool characteristics for natterjack toads (*Bufo calamita*) in different parts of the Swedish range

	West coast locality ($n = 5$) Mean ± SD	South coast locality ($n = 5$) Mean ± SD	South inland locality ($n = 5$) Mean ± SD
Breeding pools:			
Number	12 ± 13	6 ± 6	6 ± 4
Mean area (m^2)	45 ± 21	981 ± 1183	570 ± 817
Mean max. depth (cm)	37 ± 8	43 ± 7	72 ± 24
Free water surface (%)	71 ± 19	68 ± 35	76 ± 32
Shore veg. height (cm)	14 ± 3	39 ± 44	25 ± 36
Shore line substrate (%):			
Earth	<1	22 ± 22	17 ± 20
Sand	<1	12 ± 16	49 ± 36
Gravel	<1	1 ± 2	2 ± 5
Rock	74 ± 14	18 ± 40	<1
Vegetation	26 ± 14	14 ± 37	27 ± 19
Shore inclination (%):			
<30°	52 ± 14	60 ± 39	58 ± 36
30–45°	24 ± 10	6 ± 13	25 ± 33
>45°	24 ± 6	34 ± 38	14 ± 20

CLIMATE

Mean values of the climatic variables which are believed to be important for tadpole development and survival are given for the three areas in Table IV. On the west coast islands precipitation is comparatively low during spring and early summer compared with both coastal and inland southern localities. Mean air temperature during the active season is very similar in all three areas, but all coastal localities are colder in spring and warmer in autumn. The number of sunshine hours are, on average, 7% higher in the west coast islands than in the southern coastal populations and between 11 and 23% higher than in the southern inland localities.

COMPETITION, PREDATION AND HUMAN ACTIVITIES

The presence of potential competitors and predators was noted for all localities (Table V), but the importance or effectiveness of these species were not studied (see Discussion). The character of the surrounding land was also noted. Possible competition from other anuran species was limited to low numbers of the moor frog (*Rana arvalis*), on the west coast islands. In all coastal

FIG. 3. A typical natterjack toad (*Bufo calamita*) habitat from a coastal locality in the southernmost part of Sweden.

TABLE IV. Climatic characteristics for natterjack toad (*Bufo calamita*) localities in different parts of the Swedish range. (From the Swedish Meteorological and Hydrological Institute (SMHI), see Material and Methods)

	West coast locality ($n = 5$) Mean ± SD	South coast locality ($n = 5$) Mean ± SD	South inland locality ($n = 5$) Mean ± SD
Mean monthly rain (mm):			
Apr–May	29.5 ± 0	37.8 ± 2.1	34.6 ± 0.2
Jun–Aug	52 ± 0	63.8 ± 4.6	60.5 ± 1.8
Sep–Oct	60.5 ± 0	57.0 ± 3.3	56.6 ± 2.0
Mean air tempr. (°/24 hr):			
Apr–Oct	12.4 ± 0	12.4 ± 0.3	12.4 ± 0.2
Apr–May	7.8 ± 0	7.7 ± 0.5	8.4 ± 0.5
Jun–Aug	16.1 ± 0	16.0 ± 0.2	16.2 ± 0.2
Sep–Oct	11.5 ± 0	11.6 ± 0.3	10.3 ± 0.3
Mean daily sunshine (hr):			
Apr–Oct	7.6 ± 0	7.1 ± 0.3	6.6 ± 0.1
Apr–May	8.1 ± 0	7.6 ± 0.3	7.3 ± 0.02
Jun–Aug	9.2 ± 0	8.5 ± 0.4	7.9 ± 0.03
Sep–Oct	4.8 ± 0	4.5 ± 0.2	3.9 ± 0.2

TABLE V. Potential predators and competitors present on natterjack toad (*Bufo calamita*) localities in different parts of the Swedish range. The character of surrounding land is also given

	West coast locality ($n = 5$) Mean ± SD	South coast locality ($n = 5$) Mean ± SD	South inland locality ($n = 5$) Mean ± SD
Competition (%):			
Bufo sp.	0	100	1000
Rana sp.	40	100	100
Predation (%):			
Birds	0	100	100
Mammals	0	100	100
Insect larvae and adults	100	100	100
Surrounding land (%):			
Grazed	0	100	60
Arable	0	40	80
Industrial	0	0	40
Recreational	100	60	20

and inland southern localities at least one other *Bufo* species and one or more *Rana* species were common. Potential bird and mammal predators occurred in all southern localities, but none were identified with certainty during the study on the west coast island localities. Predatory insects or insect larvae were found in all localities in the three areas. Most southern localities were influenced by grazing livestock, cropping, and to some extent by industrial activities, but this was not the case in any of the west coast populations. Recreational activities took place in all west coast and in some south coast localities.

DISCUSSION

Throughout its central and north European range the natterjack seems to show a preference for dry and sandy soils. The species selects open sandy habitats in

the Netherlands (Strijbosh, 1980), is limited to dune and heathland habitats in Great Britain (*e.g.* Beebee, 1977, 1979), and is found in sandy areas in Germany (*e.g.* Mertens, 1947) and Denmark (Volsøe, 1949). The habitat in southernmost Sweden, described by Gislén & Kauri (1959) and Berglund (1976), is similar to that in other parts of the northern and cental European range. The open, unshaded and rocky habitat in the west coast islands differ markedly from what is known from this part of the distribution, and it may be more similar to some localities in southern Spain (Valverde, 1967) where natterjacks sometimes are found in very rocky terrain.

The breeding pools selected by *Bufo calamita* are usually shallow, exposed, and temporary in character with little or no vegetation (Beebee, 1979). This general description is relevant for all three areas investigated in Sweden, but certain differences exist between the west coast islands and the southern localities. The west coast breeding pools are generally smaller, more shallow and more temporary in character and have a more varying chemico-physical composition compared with the southern localities (Andrén & Nilson, 1984). Breeding in the Baltic Sea is not uncommon on several localities along the Swedish south coast (Gislén & Kauri, 1959; Berglund, 1976; own observations), but the sea water salinity in these areas is low and does not exceed 0.4%.

The climate is a very important factor influencing tadpole development and survival along the northern border of the species range. The natterjack has a very long breeding period compared to other European anurans. The prolonged egg-laying periods may be an adaptation to the use of temporary pools and to unpredictable weather conditions which strongly influence the tadpole development rate and their chance to metamorphose before the water body used dries up. This is especially true in the west coast populations where egg laying has been observed from mid May to mid August and unmetamorphosed tadpoles have been seen up to mid October (Andrén & Nilson, 1979, 1984). The breeding pools in these localities are exceptionally small and temporary in character. Rainfall in spring is lower along the west coast than in south Swedish localities, and in a study in 1980 in this area (Andrén & Nilson, 1980) it was shown that drying up of breeding pools was highest in May and early June. The low air temperature in spring in coastal areas is compensated by the relatively high number of daily sunshine hours, which is highest in the outermost archipelago at the west coast where the natterjack islands are situated. In this area the combination of small and shallow breeding pools on open, unshaded and rocky ground and the long daily sunshine period probably result in a high tadpole development rate.

Competition at different development stages may be a highly significant factor influencing the survival and distribution of natterjacks. Heusser (1972) showed by laboratory experiments that *Bufo calamita* tadpoles suffer seriously from competition with larger tadpoles of other anuran species as well as from its own species. Mathias (1971) found larvae of *Bufo bufo* and *Bufo calamita* to be present in the same pools but at different stages of development, and this was believed to be the area of greatest interspecific conflict. He also examined the food intake of adults, and certain differences in the importance of various food groups were noted. In a study of the possible competition at the adult stage of *B. bufo* and *B. calamita* on the Swedish west coast we did not find any marked difference in size or systematic possession of the prey items consumed in allopatric populations (Andrén & Nilson, 1983, in prep.). The natterjack and the common toad seemed to have an overlapping food niche, even if that of the natterjack was slightly wider including a higher number of prey taxa. Competition with other anurans is probably much higher in the southernmost Swedish localities, and here the size of adult natterjacks is similar to that in general for the species, and more similar to other sympatric toad species. Adults in the west coast islands are somewhat smaller and rarely exceed 60 mm in length (Andrén & Nilson, 1979).

Birds such as the black-headed gull (*Larus ridibundus*), the hooded crow (*Corvus corone*), the magpie (*Pica pica*) and the grey heron (*Ardea cinerea*), as well as mammals, *e.g.* the European mink (*Lutreola lutreola*), weasels (*Mustela* sp.) and the badger (*Meles meles*) are known or believed to prey on different development stages of natterjacks (Hardy, 1939; Smith, 1969; Berglund, 1976; Andrén & Nilson, 1979; Beebee, 1979). A few of these predators are found on some natterjack islands on the west coast, but none of these islands were included in the study. Many vertebrate predators on amphibians avoid bufonid tadpoles (Heusser, 1971; Cooke, 1974a; Kadel, 1975), however, direct predation does exist. Mathias (1971) found in laboratory studies that larvae of *Dytiscus marginalis*, the beetle *Colymbetes fuscus*, and the bug *Notonecta* to be efficient predators of *Bufo calamita* tadpoles. Predation by other anuran tadpoles may also take place (Beebee, 1979). Potential invertebrate predators exist in all localities included in this study.

Pollution from industry and pesticides used in agricultural areas may seriously affect breeding and development of amphibians (Ilosvay, 1977; Cooke, 1972, 1973, 1974b, 1977; Haines, 1981). Ahlén & Berglund (1980) have observed a rather drastic decrease of natterjacks in the south inland localities in Sweden and the reason for this decrease is believed to be the destruction of habitats, *e.g.* the overgrowing of shading vegetation. Bridson (1978) found that serious damage could be done to breeding pools by the trampling of livestock coming to the water to drink or cool down, and the pollution from cattle was also devastating. In many areas a balanced grazing from livestock seems to be necessary to prevent overgrowing of open natterjack habitats. The effect of recreational activities on natterjack habitats and survival has not been investigated in Sweden, but hitherto, at least on the west coast, the effects are not believed to be serious.

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THE TIMING AND DURATION OF THE BREEDING MIGRATION OF THE COMMON TOAD (*BUFO BUFO*) AT LLANDRINDOD WELLS LAKE, MID-WALES

F. M. Slater, S. P. Gittins and J. D. Harrison

Department of Applied Biology, UWIST, Llysdinam Field Centre, Newbridge-on-Wye, Llandrindod Wells, Powys

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SUMMARY

The migrations of the common toad (*Bufo bufo*) at a lake in mid-Wales were monitored daily over five breeding seasons, from 1978 to 1982. Differences in the timing and total duration of the breeding migrations between years can be accounted for largely by variations in dusk temperature each year. Relatively high dusk temperatures early in the year, however, did not initiate migrations, suggesting that another inhibitor

to movement is operative early in the year. The number of nights on which substantial toad movement occurred was constant at about 11–12 nights each year. This is possibly related to the constant size of the breeding population, and the distance which the terrestrial toads must cover to arrive at the lake.

INTRODUCTION

A study of the common toad (*Bufo bufo*) population at Llandrindod Wells Lake in mid-Wales began in 1978 and successive years data have enabled variations in the timing and duration of the breeding migration to be compared. This complements the existing literature concerning this population (Gittins, Parker & Slater, 1980; Wisniewski, Paull, Merry & Slater, 1980; Paull, Wisniewski & Slater, 1981; Wisniewski, Paull & Slater, 1981; Gittins, Steeds & Williams, 1982; Gittins, 1983a, b; Gittins, Kennedy & Williams, 1984, in press.

METHODS

Toads were collected from the road that surrounds the lake by walking around the site once every hour during the evening and night and locating the animals with the help of a powerful hand lamp (Gittins *et al.*, 1980). As it was not possible to determine with any certainty the direction in which the toads were moving, the term migration, used here, includes movements both to and from the lake. Dusk air temperature was recorded on each visit to the lake.

For each year, days have been numbered consecutively using January 1 as day 1. Monitoring at the lake prior to the migration began about day 60 (1 March) and finished when several days had elapsed with few, if any, captures following the main outward movement.

The timing of the migration is referred to in terms of the median date of activity, which is the date by which 50% of the total number of animals captured that year have been counted. The total duration of the migration each year is the interval between the first and last capture, and includes nights on which no captures were made. The number of nights on which substantial toad movement occurred (*i.e.*, "the active duration") is defined as the number of nights on which more than 75 individuals were collected, as this amounts to approximately 1% of the breeding population (Gittins, 1983b).

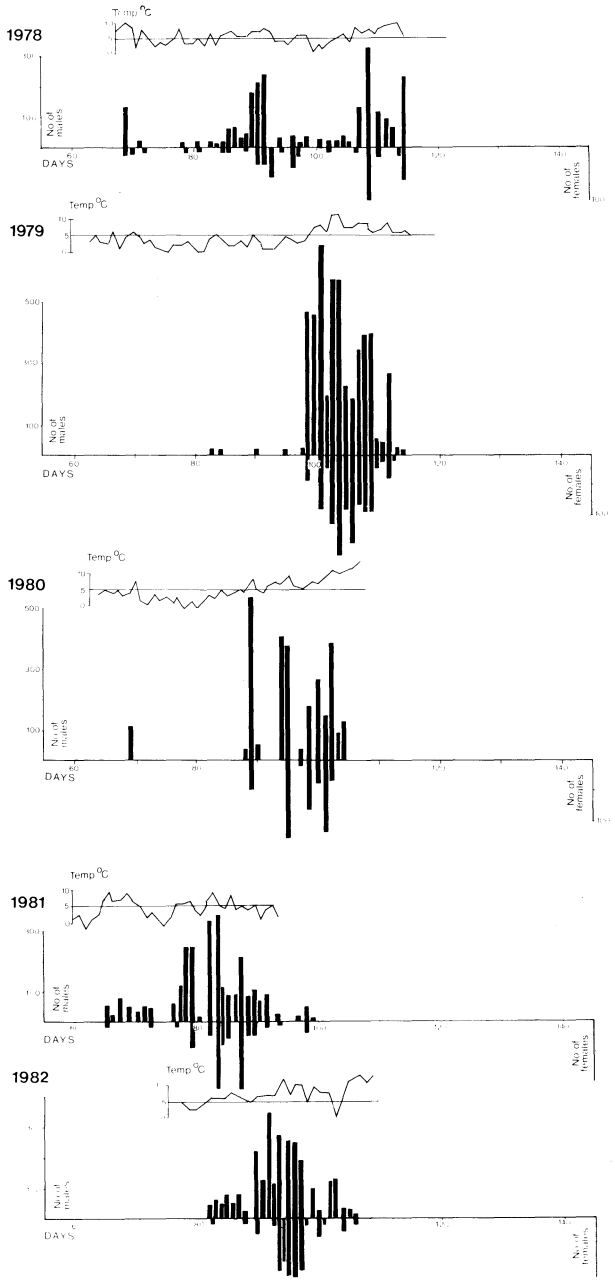

FIG. 1. Toad migrations at Llandrindod Wells Lake between 1978 and 1982. Numbers plotted for any date are those caught in the first two collecting circuits of the lake. Days are numbered consecutively from January 1st. Numbers of males are shown above and numbers of females below the horizontal axis. Dusk air temperature is plotted above the animal data for each respective year.

RESULTS

At Llandrindod Wells Lake, there are clearly differences between years in the onset of the migratory period for toads (Fig. 1). Similarly, the median dates of actively vary over the 5-year period by 22 days for males and 24 days for females (Table 1). Although the total duration of the migration varies from 25 days (1982) to 46 days (1978), the number of nights on which substantial toad movement occurred (the active duration) was remarkably constant over the 5-year period at 11–12 days (Table 1).

Variations in the patterns of toad movement between years can be accounted for largely by fluctuations in temperature, as little toad activity occurred when dusk air temperatures fell below 5°C (Fig. 1).

Every year, the onset of the main female migratory period was significantly later than that for males (Kolmogorov–Smirnof two sample test, $P < 0.01$).

TABLE I. The timing and duration of the toad migrations at Llandrindod Wells Lake, 1978–1982. Median date of activity is the day by which 50% of the total number of toads collected that year were counted. Total duration is the interval between the first and last capture. Active duration is the number of nights on which >75 toads were collected.

Year	Median dates of activity males	Median dates of activity females	Total duration (days)	Active duration (days)
1978	105 (15 April)	109 (19 April)	46	12
1979	104 (14 April)	105 (15 April)	34	12
1980	96 (5 April)	97 (6 April)	35	12
1981	83 (24 March)	85 (26 March)	34	11
1982	93 (3 April)	95 (5 April)	25	11

DISCUSSION

Variations in evening temperature may account for much of the variation between years in the onset and total duration of the toad migrations at Llandrindod Wells Lake. The results conform with previous studies and suggest the presence of a minimum threshold temperature for toad activity. Recent work on the Llandrindod toad population has determined this threshold at around 3–4°C (Gittins *et al.*, 1980; Wisniewski *et al.*, 1981). Other studies have recorded higher critical temperatures for toad activity, for example, Heusser (1969) estimated it as 5–6°C for a Swiss population and Frazer (1956) found that toads at St Ives, Cornwall, moved to the breeding pond when the air temperature was about 7°C, while an Exmouth population had a higher threshold temperature. The threshold temperatures for toad activity, therefore, may well be population specific as Frazer (1966) suggested.

There is little evidence to suggest that dusk air temperatures prior to about day 60 have any effect on the onset of toad migration. In some years, evening temperatures in mid or early February exceeded 5°C, but visits to the lake revealed little sign of toad activity. This suggests that another inhibitor to movement is operative early in the season. The nature of this inhibitor is not known: it may be related to seasonal endocrinal changes such as those described for the common frog (van Oordt & Lofts, 1963; Lofts, Wellen & Benraad, 1972; Julich, Haider, Passia & Goslar, 1982). These changes may be regulated by an internal clock and/or photoperiod, similar to the conditions controlling spermatogenesis in salamanders (Werner, 1969).

The consistent length of the active toad migration may be related to the constant size of the breeding population (Gittins, 1983b) and the distance from which the toads migrate. It seems that 11–12 warm evenings are adequate to allow the majority of the breeding population to move from their terrestrial habitats into the lake.

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OSMOTIC FRAGILITY OF RED BLOOD CELLS IN THREE SPECIES OF AMPHIBIANS

GAD DEGANI

Galilee Technological Center, Chemical and Microbiological Laboratories, Kiriat Shmona 10200, *Israel*

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SUMMARY

The fragility of red blood cells in three different amphibians (*Bufo viridis*, *Rand ridibunda* and *Salamandra salamandra*) was studied.

The semi-aquatic species *R. ridibunda* shows a lower fragility of red blood cells than the terrestrial species, *B. viridis*. The blood cells of *R. ridibunda* were acclimated in 90 mM/l NaCl, 50% haemolysis was 27 ± 3 mM/l. By comparison, in red blood cells of *B. viridis* which were acclimated to the same solution, 50% haemolysis was 33 ± 4 mM/l.

The red blood cell fragility of *B. viridis*, *R. ridibunda* and *S. salamandra* is correlated to osmotic concentration of the solution to which the red blood cells acclimated before the fragility was tested.

The 50% haemolysis of *B. viridis*, both before and after dehydration, was higher than the 50% haemolysis of *S. salamandra*, under the same conditions.

INTRODUCTION

The toad *Bufo viridis* is found throughout Israel, including areas of arid habitats in the Negev (Warburg, 1971). It burrows under ground during the hot and dry summer. Degani, Silanikove & Skolnik (1981) found that during burrowing in the soil, in the summer and under laboratory conditions, the plasma concentration was greatly elevated (1400 mOsm/kg^{-1}).

The frog *Rana ridibunda* is found in permanent ponds, springs and streams, where it stays in or near the water (Warburg, 1971). The plasma concentration is not as high during dehydration or acclimation to salt water as that of *B. viridis* (Katz, 1973).

Salamandra salamandra is a terrestrial Urodele which successfully adapted to xeric habitats (Degani & Warburg, 1978); Degani & Mendelssohn, 1979), and shows better adaptation to terrestrial life than *R. ridibunda*, but is less adaptive than *B. viridis*. Salamanders from xeric habitats (Mount Carmel and Galilee) can tolerate plasma concentrations of 770 mOsm/kg^{-1} (Degani, 1981a). Salamanders from moist habitats (Tel Dan) are less adaptive to terrestrial life and show a lower tolerance to NaCl solution than salamanders from xeric habitats (Degani, 1981b).

The amphibian skin integument plays an important role or osmoregulation. Amphibians in air lose water by evaporation, in fresh water they gain water by osmosis and salts by diffusion (Bentley & Main, 1972). The water passes through the skin directly to the blood plasma, and decreases the plasma concentration. *Chiromantix xerampelina* was readily able to absorb water across its protected ventral skin, but lost water very slowly from its dorsal surface. The ventral skin in other amphibia was also found to be very important in the absorption of water. This area of skin is very permeable (McClanahan & Baldwin, 1969) and very rich in blood vasculation (Christensen, 1974; Czapek, 1965).

Water uptake through the pelvic integument in *Bufo punctatus* showed that about 70% of this specialized area of vascularization is more dense than the rest of the skin (Christensen, 1974). During rehydration, the water passed directly into the plasma, thus diluting the plasma concentration (Bentley & Main, 1972; McClanahan & Baldwin, 1969) and exposing the red blood cells of the amphibia to a different osmotic concentration. The successful adaptation of adult amphibia to arid environments (McClanahan, 1972; Delson & Whitford, 1973; Degani, 1981a; Degani *et al.*, 1981) is aided by the elevation of their total plasma osmotic concentration. In aquatic amphibians, the plasma osmotic concentration could not elevate their total plasma concentration, as can be found in terrestrial forms. Compared to other classes, for example, mammals (Livne, Kuiper & Meyerstein, 1972; Perek, 1966; Perek, Hart & Perri, 1963; Yagil, Morian & Myerstein, 1974; Chosniak & Skolnik, 1977), little is known of red blood cell haemolysis (RBCH) of amphibia (Zeidler, 1979).

The purpose of this research is to study the osmotic fragility of the red blood cells and the influence of the elevated plasma concentration of three amphibians.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Adult *Bufo viridis* (weight 20–60 g) were collected from the Mount Meron area during summer (June–July) as previously described by Degani & Warburg, (1978). Adult *Salamandra salamandra* weight 25–90 g) were collected at the beginning of winter (Degani & Warburg, 1978), from xeric habitats of the Mount Meron area. The toads and salamanders were kept in plastic containers on a 12 hr light and 12 hr dark cycle at 16 ± 0.5°C on moist soil, and were fed on larvae of *Musca domestica* and on *Lumbricus sp.*

Adult *Rana ridibunda* (weight 20–80 g) were collected from Sasa Pond, as described previously be Degani & Mendelssohn (1979), and were kept in plastic containers with water, 2 cm deep at 16 ± 0.5°C, on a 12 hr light and 12 hr dark cycle.

Experiments

Toads (weight 20–30 g) and salamanders (weight 20–30 g) were allowed to dehydrate 15–20% of their body weight at 25°C and 0–10%RH, as described previously by Warburg & Degani (1979).

Sampling

Blood was obtained from hydrated *B. viridis*, *R. ridibunda* and *S. salamandra* and dehydrated *B. viridis* and *S. salamandra* (15% body weight). The blood samples were taken by heart puncture with a 1 ml syringe previously washed with lithium heparin, as described previously by Degani (1981a). The blood samples were immediately transferred to centrifugal tubs and centrifuged for 10 min at 300 r.p.m.

Osmotic Fragility

The osmotic fragility of the red blood cells was determined following the method described by Livne *et al.* (1972) and modified by Chosniak & Skolnik (1977). The osmotic fragility of the red blood cells was determined in two groups of cells (1) Red blood cells after centrifuge, (2) Red blood cells that, after separating, were washed three times with different concentrations of NaCl solution (60 mM/l and 90 mM/l), and then acclimated for 15 min in the solutions before red blood cell haemolysis was tested.

RESULTS

Rana ridibunda was shown to have a lower fragility of red blood cells than the *Bufo viridia* (Table I). The red blood cells of *B. viridis* which had been acclimated in a solution of 90 mM/l NaCl, showed a high haemolysis in a solution of 32 mM/l NaCl (47%) compared to *R. ridibunda*, which was found to have only 16% haemolysis in the same solution (Table I), and the difference is significant ($P < 0.05$; *t*-test). No significant difference was found between the fragility of red blood cells of *B. viridis* and *R. ridibunda* which has been acclimated in a solution of 60 mM/l NaCl.

The 50% haemolysis of *B. viridis* before and after dehydration was higher than the haemolysis of the *S. salamandra*. The plasma concentration of *B. viridis* was found to be higher than that of the *S. salamandra*. Fifty percent of the red blood cells of the *S. salamandra* specimens from xeric habitats haemolysed in a solution of 20 ± 2 mM/l NaCl before dehydration, and 50% of the red blood cells haemolysed in a solution of 30 ± 3 after dehydration of about 15% of the body weight (Table 2). A similar situation was found in comparing 50% haemolysed red blood cells of *B. viridis* before and after dehydration. The plasma concentrations of *B. viridis* were higher than those of the *S. salamandra* (Fig. 1).

The difference between the 50% haemolysed red blood cells of *R. ridibunda* and *B. viridis* was found to

TABLE I. Osmotic fragility of *Bufo viridis* and *Rana ridibunda* red blood cells acclimated to 60 and 90 mM/l NaCl. In all cases $N = 5$

NaCl concentration (mM/l)	Percent haemolysis ± SD *Bufo viridis*	*Rana ridibunda*
Acclimation to 60 mM/l:		
2	100 ± 3	99 ± 4
13	87 ± 25	69 ± 23
23	74 ± 31	35 ± 19
32	38 ± 22	12 ± 2
44	18 ± 10	10 ± 6
Acclimation to 90 mM/l:		
2	100 ± 4	100 ± 1
13	96 ± 4	96 ± 3
23	89 ± 5	63 ± 18
32	47 ± 23	16 ± 11
42	8 ± 3	11 ± 6

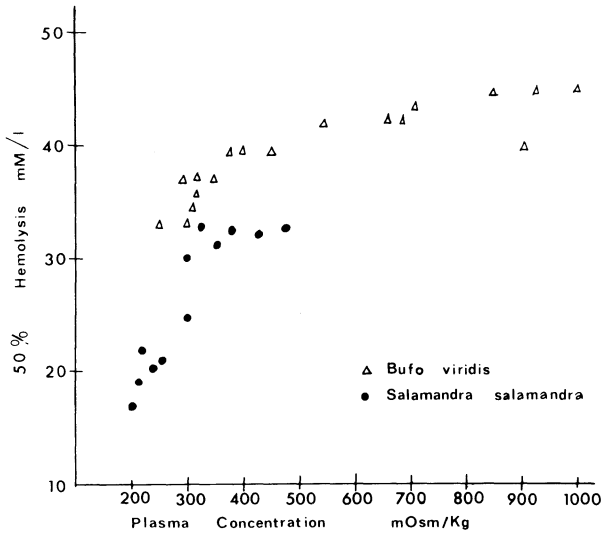

FIG. 1. The 50% haemolysis of red blood cells as a function of plasma osmolality from *Salamandra salamandra* and *Bufo viridis*.

TABLE II. Fifty percent haemolysis of red blood cells of *B. viridis*. *R. ridibunda* and *S. salamandra* in different plasma concentrations

Animals	*N*	Plasma concentration mOsm/kg^{-1}	50% haemolysis mM/l^{-1}	*P* (*t*-test)
Bufo viridis				
Hydrated	5	320 ± 40	33 ± 5	<0.001
Dehydrated (15% body weight)	6	753 ± 112	42 ± 2	
Salamandra salamandra (Xeric habitat, Galilee)				
Hydrated	5	228 ± 10	20 ± 2	<0.001
Dehydrated (15% body weight)	5	383 ± 36	30 ± 3	

TABLE III. Fifty percent haemolysis of red blood cells in the different concentrations of NaCl

		Medium concentration N mM/l^{-1} (NaCl)	50% haemolysis mM/l^{-1}	P (t-test)
Bufo viridis	5	90	33 ± 4	<0.05
Rana ridibunda	5	90	27 ± 3	
Bufo viridis	5	90	18 ± 3	N.S.
Rana ridibunda	5	60	17 ± 5	

be significant only in cells that acclimated to 90 mM/l NaCl, and not in the cells that acclimated to 60 mM/l NaCl (Table III).

DISCUSSION

The osmotic stability of red blood cells of three different species of Amphibia (*Bufo viridis*, *Rana ridibunda* and *Salamandra salamandra*) was found to be a function of plasma or media concentration to which the red blood cells were acclimated before haemolysis was examined. The stability of red blood cells was found to be in opposite correlation to the osmotic concentration. For this reason, it is impossible to compare the stability of red blood cells if they were not found before in a similar solution or plasma concentration.

Another very important conclusion is that the red blood cells of Amphibia can adapt to different solutions. This factor was found in the three species (*B. viridis*, *R. ridibunda* and *S. salamandra*) and may possibly occur in other amphibia too.

These results support the hypothesis that after dehydration, or during long periods of burrowing in soil (McClanahan, 1972; Degani *et al.*, 1981), when the plasma concentration rise (*e.g.* Degani, 1981a, Warburg, 1972; Bentley, 1972), the red blood cells adapt to high concentration. During rehydration, the water dilutes the plasma slowly, and the red blood cells acclimate to low plasma concentration. Zeidler (1978) found that the 50% haemolysis of red blood cells of *Xenopus laevis* (an aquatic species) maintained in tap water, was 20 mM/l NaCl, similar to the findings in hydrated *S. salamandra*, or the red blood cells of *B. viridis* and *R. ridibunda* that acclimated to 60 mM/l NaCl. However, the difference between 50% haemolysis of red blood cells of *B. viridis* and *R. ridibunda* was found to be significant after the red blood cells were acclimated to 90 mM/l. Terrestrial *B. viridis* can tolerate high plasma concentration after adaptation to saline solution (Katz, 1973), or during long periods of burrowing in the soil (Degani *et al.*, 1981). The difference between 50% haemolysis of red blood cells of *B. viridis* that acclimated to two solutions (60 mM/l NaCl and 90 mM/l NaCl) before haemolysis was studied, was greater (15%) than the difference of 50% red blood cells of *R. ridibunda* (10%) that were acclimated before haemolysis was studied under the same conditions. It is therefore possible that the red blood cells of *B. viridis* are better adapted to changes to different plasma concentrations.

The change of osmotic fragility of red blood cells of *Salamandra salamandra* from xeric habitats is correlated to osmotic concentration of the plasma. Similarly, a change of osmotic fragility in the red blood cells of *B. viridis* (Fig. 1) was found. The plasma concentration of *B. viridis* during dehydration can reach to higher osmolality than the plasma concentration of *S. salamandra*. The results found in this study were in agreement with other papers (Katz, 1973; Degani, 1981a, b; Degani *et al.*, 1981). The comparison between the fragility of red blood cells of *S. salamandra* and *B. viridis* that was found in plasma concentration between 300–400 mOsm/kg (Fig. 1), show that the fragility of red blood cells of *B. viridis* is higher than the fragility of red blood cells of *S. salamandra*. It is possible to explain these results by the fact that the red blood cells of terrestrial amphibia such as *B. viridis*, that adapted to arid habitats, are found in higher plasma concentration than the red blood cells of *S. salamandra*, and are therefore not exposed to hypo-osmotic pressure.

The only place that the red blood cells could be exposed to hypo-osmotic pressure is in the pelvic integument, where the vascularization is denser than the rest of the skin, and water passes through the skin to the plasma (Christensen, 1974). It is possible that, when the red blood cells found in the capilliary walls prevent haemolysis by holding the cell membrane, and following this, when the red blood cells pass to the bigger vascular, the plasma concentration is higher and the red blood cells adapt to this concentration.

The osmotic fragility of amphibian red blood cells is quite different from that of human cells (Zeidler, 1979) or Bedouin goat or other ruminants (Perek *et al.*, 1963; Bianca, 1970; Chosniak & Skolnik, 1977). All of these types of blood cells are non-nucleated.

Fifty percent haemolysis is found between 35–65 mM NaCl from armoured catfish (*Pterygoplichthys*), flounder (*Paralichthys pethostigmus*), spotted Gar (*Lepidosteus productus*), sea catfish (*Galeichys felis*) and *Miropogan undulatus* (Ezell, Sulya & Dodgen, 1969; White & Brown, 1981). This is higher than was found in amphibians in this study.

This study supports the hypothesis that the highest stability of red blood cells is found in amphibian species with low plasma concentration (200–500 mOsm/kg) such as the semi-aquatic *R. ridibunda* (Katz, 1973). A lower stability of red blood cells was found in terrestrial *S. salamandra*, whose plasma concentration varied between 200–700 mOsm/kg (Degani, 1981a, b). The lowest stability of red blood cells was found in *B. viridis*, a terrestrial species, whose plasma concentration varied between 300–1400 mOsm/kg (Katz, 1973; Degani *et al.*, 1981).

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THE EMERGENCE OF POSTMETAMORPHIC SMOOTH NEWTS FROM A POND IN SOUTHERN ENGLAND

PAUL A. VERRELL

Animal Behaviour Research Group, The Open University, Milton Keynes MK7 6AA

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SUMMARY

Emergence of 395 postmetamorphic smooth newts (*Triturus vulgaris*) from Yrrell Pond in 1983 extended from 22 August until 19 December, with a peak in September–October.

INTRODUCTION

There are no quantitative data available on the emergence of postmetamorphic smooth newts (*Triturus vulgaris*), or efts, from water onto land. Reviewing rather fragmentary and often anecdotal information, it seems that metamorphosing larvae and emerged efts have been found from mid June to late September in England (Bell, 1977; Creed, 1964), late June to late August in Poland (Kowalewski, 1974) and early August to late September in Sweden (Hagstrom, 1974). In some cases, efts left the water at the same time as post-breeding adults (Kowalewski, 1974); in others, there was less, if any, overlap (Bell, 1977).

In this paper, quantitative and temporal data on the emergence of efts from a single breeding site in

southern England are presented, together with estimates of productivity and survivorship in 1983.

MATERIALS AND METHODS

Yrrell pond, near Soulbury, Buckinghamshire, has a surface area of about 120 m^2 and is a deep, permanent pond situated on private farmland. It is a breeding site for *T. vulgaris*, *T. cristatus* (crested newt), *Bufo bufo* (common toad) and *Rana temporaria* (common frog). From 28 February 1983 until mid-April 1984, the pond was surrounded with a polythene drift fence, buried to a depth of at least 5 cm and extending above ground to a height of 25 cm. Every two or three days, the ground between the edge of the pond and the inside of the fence was carefully searched for emerged efts. The snout-vent length of each eft found was measured with a ruler (to the nearest 0.5 mm). In addition, smooth newt larvae in the water were captured by dipnetting. The snout-vent length of each larva was measured by placing it in a container on graph paper marked in 1 mm graduations (to the nearest 0.5 mm). Data on adult smooth newts and other amphibians present were collected in separate studies of their ecology.

RESULTS AND DISCUSSION

Emerged smooth newt efts were first found on the inside of the drift fence on 22 August; the last captured in 1983 was on 19 December. A total of 395 efts were captured during this 17-week emergence period, the majority in the months of September and October (Fig. 1). This period completely overlapped that of the emigration of post-breeding adults, which lasted from early June to late December.

The median snout-vent length of the efts captured in 1983 was 19 mm, with a range of 11 to 27 mm. Efts found early in the 17-week period were few in number, but tended to be larger than those captured later (Fig. 2). The smallest eft found was 11 mm, presumably the smallest length at which metamorphosis can occur. Dipnetting of the pond was conducted for the first 11 weeks of the emergence period, between 09:00 and 11:00 hr; 54 larvae were captured, at various stages of development. The median length of these larvae was 12 mm, with a range of 9 to 14.5 mm. Efts often clambered onto the shore from the water during episodes of dipnetting; whether emergence is diurnal in the smooth newt, or efts simply leave the water if disturbed, is not clear.

Bell & Lawton (1975) observed three cohorts of larvae in a pond near Oxford, England, hatched from three cohorts of eggs. My data on the phenology of emergence do not provide evidence for the presence of distinct cohorts of efts (Fig. 1). This probably reflects a single, more or less synchronous period of oviposition in the months of May and June. The timing of ovulation in adult females was determined by gently pressing the bellies of all females captured in the pond; only females with eggs in their oviducts extrude eggs from the cloaca during this treatment (Verrell, 1984).

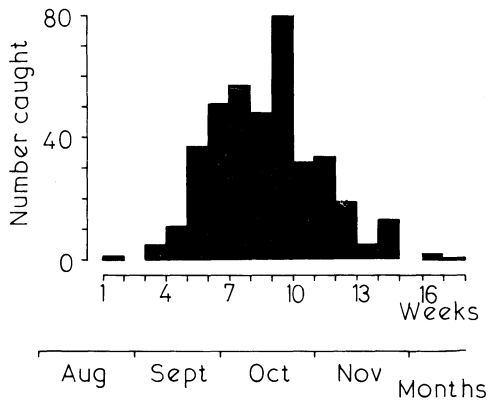

FIG. 1. The timing of emergence of efts over a 17-week period.

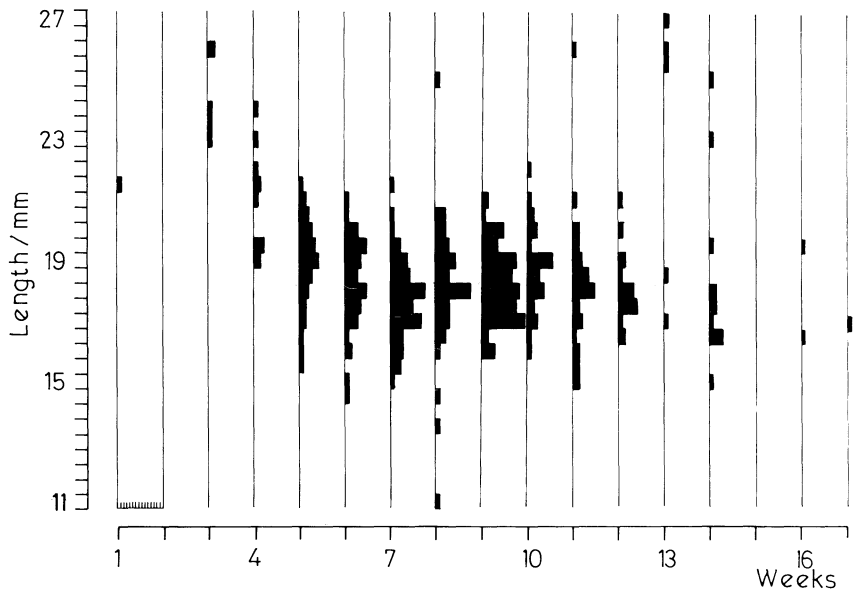

FIG. 2. Length-frequency histograms for efts emerging in each week of a 17-week period.

In March and April 1984, 13 smooth newt larvae (median length 18 mm, range 16 to 24 mm) were captured in the water, and 27 efts (median length 24 mm, range 19 to 31 mm) were captured on the inside of the drift fence. The relatively large size of these animals and their very early appearance in the year (the immigration of adult smooth newts was very sparse during these months) both suggest that they may have been the products of matings in 1983 which had overwintered in the water. Larval overwintering of this type does not seem to be a common feature of published studies of smooth newt biology (but see Bell & Lawton, 1975); however, the early emigration of overwintered juveniles has been reported for some populations of the North American red-spotted newt, *Notophthalmus viridescens* (Healy, 1975).

No efts were observed climbing over the fence, none were found in the grass adjacent to the outside of the fence and none were found against the buried part of the fence when it was removed; I thus assume that few, if any, efts evaded capture. It is thus reasonable to estimate the productivity of the population in 1983 and the survivorship from egg to emerged eft. These may be minimal rather than absolute estimates, with unknown margins of error. Two hundred and twelve adult female newts were captured on the outside of the fence between the beginning of March and the beginning of July, and all were toe-clipped before they were released into the pond. During sessions of dipnetting, 560 females were captured, and using a simple Lincoln Index on mark-release-recapture data, I estimate that approximately 664 females visited the pond in 1983. Forty-eight females were captured as they migrated to other ponds in Buckinghamshire, and the total number of yolked oocytes in their ovaries counted. These dissections yielded a mean fecundity of 262 ova per female, with a range of 94 to 501 ova. Thus, at Yrrell pond in 1983, about 400 efts emerged from some 173 968 ova, giving a productivity of 0.6 efts per female and a probability of survival from egg to eft of 0.0023. The cause(s) of mortality of eggs, larvae and efts was not addressed in this study; however, the pond contained and was visited by a number of predatory invertebrate larvae and vertebrate predators (carp, smooth and crested newts, heron and shrew).

Bell & Lawton (1975) estimated survivorship from egg to eft as 0.09 for their population near Oxford, capturing efts with pitfall traps placed around part of their pond. Assuming that equal numbers emerged from all parts of the pond, these workers calculated total eft emergence by multiplication. This may not be a valid assumption, for at Yrrell pond, the highest numbers of efts were captured adjacent to those areas of the pond which were most heavily weeded and shallow.

In summary, the data presented above show that smooth newt efts emerge from the water over a relatively long period of time, some perhaps overwintering there to emerge in the following spring. The productivity of the smooth newt population appears to have been low in 1983, with only a tiny proportion of eggs giving rise to emerged efts. Various workers have found that density-dependent (*e.g.* intraspecific competition) and -independent (*e.g.* climatic fluctuations) factors greatly influence the young stages of amphibians (for example, the longterm study of *Ambystoma tigrinum* of Semlitsch, 1983). My data were obtained in a single year at just one breeding site, and probably represent only a part of the variability that may exist in the biology of the early stages of the smooth newt.

ACKNOWLEDGEMENTS

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 6, pp. 433–434 (1985)

AUTUMNAL MIGRATION AND AQUATIC OVERWINTERING IN THE COMMON FROG, *RANA TEMPORARIA* (SHORT NOTE)

P. A. VERRELL AND T. R. HALLIDAY

Animal Behaviour Research Group, The Open University, Milton Keynes MK7 6AA, U.K.

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Common frogs (*Rana temporaria*) are commonly observed migrating in large numbers to breeding ponds shortly before they spawn in the spring (Frazer, 1983). During a seven-year-study of frog mating behaviour at a pond near Oxford, one of us (T.R.H.) has observed that the numbers of animals recorded during spring migration cannot account wholly for the numbers present in the pond during spawning. The ratio of males to females captured while approaching the pond is very close to 1 : 1, but within the pond there are clearly many more males than females. This suggests that a substantial number of males are already in the pond before spring migration begins. This is supported by Hazelwood (1969) who found a large number of male frogs buried in the mud at the bottom of a pond that was drained during the winter.

In a study, primarily designed to investigate newt reproductive biology, we have encircled a small pond in Buckinghamshire with a drift fence and pitfall traps. Between 12 March and 19 April 1983, 14 male and 9 female frogs were captured on the outside of the fence as they migrated to the pond prior to spawning. Between 30 March and 2 May 1983, 17 males and 7 females were found on the inside of the fence, apparently leaving after spawning. No further frogs were captured until the period 27 September to 28 November 1983, when 23 males and 3 females were captured on the outside of the fence. The females were nearly as gravid as those observed during the spring migration and the males possessed well-developed nuptial pads, grey in colour, on their thumbs. They thus appeared to be in reproductive condition, or very nearly so.

Although the numbers of animals involved in this autumn migration are quite small, they do exceed those intercepted during the spring migration, suggesting that a large proportion of the breeding population overwinters in the pond. Our observations also suggest that autumn migrants are predominantly males, though not exclusively so.

Over the autumn period when we captured the frogs, we also captured a number of crested newts (*Triturus cristatus*). All these animals, 9 males and 7 females, were in reproductive condition, resembling animals caught during spring migration in terms of the texture of their skin and the development of their secondary sexual characteristics. We have not, however, captured individuals of the other two species that breed in this pond, the toad (*Bufo bufo*) and the smooth newt (*Triturus vulgaris*), moving towards the pond at this time of year. The smooth newt has been reported to show autumn migration elsewhere in England (Bell, 1977) and in West Germany (Blab & Blab, 1981). Toads have been found moving towards their breeding site in the autumn in Switzerland, although they spent the winter on land near the pond and did not enter the water (Heusser, 1969).

What adaptive advantage might frogs and other amphibians gain by overwintering in the water? Such behaviour may entail risks; prolonged freezing of the water can result in death by suffocation if dissolved oxygen levels fall too low, a phenomenon known as winterkill (Bradford, 1983). One possible advantage may relate to thermal buffering. Fluctuations in temperature will tend to be less marked in deep water than on land (Moss, 1980), so that animals overwintering in water would not experience large variations in metabolic rate. Another possible advantage may arise from being at the spawn site before other animals arrive in the spring. In frogs, as in toads Davies & Halliday, 1979), males compete for sexual access to females and, if mating success were higher for those males that capture females first, sexual selection could favour overwintering in the pond.

The crested newts that we have captured migrating in autumn have been significantly smaller than those caught in spring ($t = 4.9$, $P < 0.005$), suggesting that they may be young animals coming to breed for the first time. The size distribution of the autumn migrating frogs was not significantly different from that of the spring migrants ($t = 0.9$, $P > 0{\cdot}10$), however, and thus they do not appear to represent any particular age class.

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This study was supported by a grant from the Open University's Research Committee. We are very grateful to Mrs Yrrell for allowing us to work at her pond.

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 6, pp. 434–435 (1985)

ARE MARINE IGUANA TAILS FLATTENED? (SHORT NOTE)

C. RICHARD TRACY[1] AND KEITH A. CHRISTIAN[2]

[1]*Department of Zoology, Colorado State University, Fort Collins, CO 80523 and* [2]*Department of Biology, University of Puerto Rico, Rio Piedras, Puerto Rico* 00931

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Charles Darwin (1883) reported that the marine iguanas of the Galapagos Islands had morphological characteristics that he interpreted to be adaptations for swimming. These characteristics included partially webbed feet and a flattened tail. However, a recent report questioned these observations stating that the tails of marine iguanas do "not differ greatly from ... *Iguana iguana*" (Dawson, Bartholomew & Bennett, 1977). In this note, we present observations on the size and shape of the tails of marine, land, and green iguanas to bear upon possible morphological differentiation in response to selective forces for efficient swimming.

Measurements of tail and body dimensions were made on land iguanas (*Conolophus pallidus*) from Isla Sante Fe, Galapagos; marine iguanas (*Amblyrhynchus subcristatus*) from Isla Santa Fe and Isla Santa Cruz, Galapagos; and on green iguanas (*Iguana iguana*) from the Denver Zoo, Colorado. Tail and body lengths were measured with a fibreglass tape to the nearest millimetre. Tail heights and widths were measured to the nearest millimetre with a caliper. Only lizards that appeared to have no damage to their tails were measured. All measurements of tail height and tail width were taken at a point two-thirds of the tail length distal to the body, and all measurements of tail height included the height of dorsal spines.

The ratio of tail length to snout-vent length for marine iguanas was unrelated ($F[1, 16] = 0.52$, $P > 0.2$) to the size of lizards, whereas the same ratio for land iguanas significantly decreased ($F[1, 17] = 90.4$, $P < 0.0001$) with respect to the size of the animals (Fig. 1). This suggests that tail and body of marine iguanas grow at the same rates, whereas the tails of land iguanas grow significantly slower than the bodies of these lizards.

The ratios of tail height to tail width (Fig. 2) in land and green iguanas are indistinguishable and unrelated to the size of the lizards ($F[1, 17] = 2.3$, $P = 0.13$ for land iguanas). Furthermore, the ratios were very close

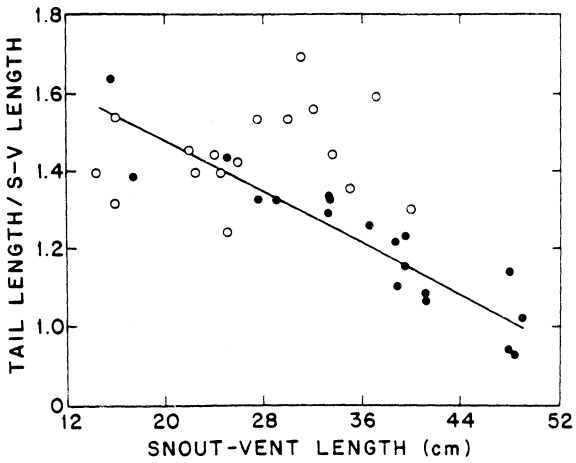

FIG. 1. The ratio of the length of the tail divided by the snout-vent length of the lizard as a function of the snout-vent length. Closed circles and regression line are for land iguanas, and open circles are for marine iguanas.

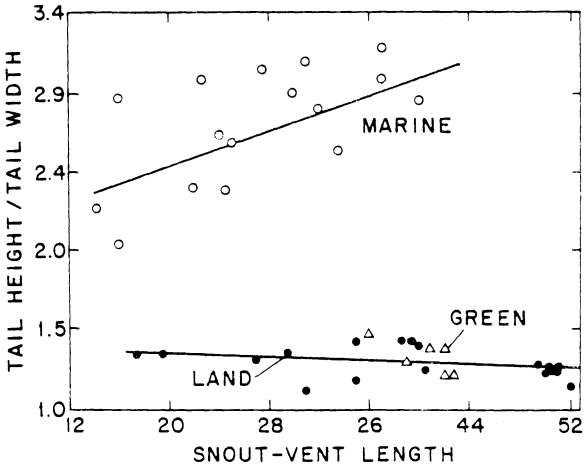

FIG. 2. The ratio of the height of the tail divided by the width of the tail as a function of the snout-vent length of the lizard. Open circles = marine iguanas; closed circles = land iguanas; triangles = green iguanas.

to unity indicating that the tails were only slightly taller than they were wide. However, the tails of marine iguanas were 2–3.2 times taller than they were wide, and adult lizards had significantly more laterally-flattened tails than juveniles ($F[1, 15] = 8.7, P < 0.01$).

There can be no doubt that tails of Galapagos marine iguanas are laterally flattened as first noticed by Darwin (1883). It also appears that adults have flatter tails than juveniles which are known to swim only rarely (Dawson *et al.*, 1977). It seems likely that this flattened tail is an adaptation for swimming since, in addition to it being the appropriate shape for a swimming fin for an animal with muscles for lateral undulations, it is also different from the tail morphology of the terrestrial land iguana and the arboreal green iguana.

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 6, pp. 435–436 (1985)

VIABILITY OF THE EGGS OF THE DAY-GECKO *PHELSUMA SUNDBERGI* EXPOSED TO SEA WATER (SHORT NOTE)

ANDREW S. GARDNER

Department of Zoology, University of Aberdeen[1]

(*Received* 7 *July* 1984)

INTRODUCTION

Geckos are amongst the most successful lizards at colonizing oceanic islands. For example, there are eleven species of geckos out of eighteen lizard species found in the Republic of Seychelles (Gardner, 1984), and twelve out of seventeen lizard species from the Mascarene islands are geckos (Vinson & Vinson, 1969; Cheke, 1982). At first sight, this is surprising in view of the relatively thin and delicate nature of gekkonid skin. Indeed, it is not known how well adult geckos are able to survive rafting, and observations in the Seychelles suggest that day-geckos (*Phelsuma* Gray 1825) avoid trees exposed to sea spray-drift.

However, there are several features of the egg stage of geckos which may be favourable for promoting successful overwater colonization. Firstly, they have a dense, calcareous eggshell which gives the egg a considerable ability to withstand dessication. Gecko eggs are frequently placed in exposed and dry sites, yet remain fully viable. Secondly, gecko eggs tend to have long incubation periods, usually over fifty days. *Phelsuma sundbergi* eggs have an incubation period of approximately seventy-five days, at a temperature of 24–29°C which is similar to that in natural incubation sites (Gardner, 1984). Thirdly, many gecko species tend to lay eggs in communal egg caches. *Phelsuma sundbergi* frequently deposit their eggs in the hollow trunks of coconut trees. The twenty-seven eggs used in this experiment, were obtained from a single hollow in a rotting coconut trunk. Moreover, coconut trees frequently overhang the beach. If geckos do colonize islands by rafting at the egg stage, the eggs must remain viable after immersion or contact with sea water. To test this with *Phelsuma* I carried out the following simple experiment.

METHODS

Phelsuma lay a clutch of two eggs, which are usually cemented together as a pair. Twenty-seven *Phelsuma sundbergi* eggs (12 pairs and 3 singles) of assorted ages

[1] Present address: *The Galton Laboratory, Department of Genetics and Biometry, University College, London NW1 2HE.*

were collected in a hollow coconut trunk near the sea at Anse Kerlan, Praslin, Seychelles. These were graded for freshness by eye, from pink, translucent, fresh eggs to grey, opaque, old eggs. Eggs were subjected to total immersion under 5 cm of sea water at 26.7–28.5°C. The eggs were removed after a given time, dried, labelled and incubated in a plastic box on a bed of lightly moistened sand. The box was checked daily for hatchlings, which were removed along with the egg-shell fragments. One pair of newer eggs and one pair of older eggs were immersed for 5 min, 60 min, 24 hr and 1 week. Single pairs of intermediately aged eggs were immersed for 1 min, 30 min and 6 hr. The remaining eggs were not immersed as controls. All the eggs sank readily.

RESULTS

All the eggs immersed for 6 hr or less hatched successfully, as did all the controls. The fresher eggs immersed for 24 hr hatched, whereas the older ones did not. None of the eggs immersed for one week hatched.

DISCUSSION

It is apparent that *Phelsuma* eggs are remarkably tolerant to sea water immersion. A somewhat similar experiment has been carried out by Brown & Alcala (1957) using the eggs of the Gekkonid species *Cosymbotus platyurus*, *Gehyra mutilata* and *Hemidactylus frenatus*. Rather than total immersion, they continuously wetted the eggs with sea water using wicks, and found that, although the experimental eggs had a lower viability than the controls, some remained viable after eleven days.

Two observations suggest that the factor limiting viability in the present experiment may be the gaseous exchange rates of the embryos, rather than osmotic effects. Firstly, 24 hr of immersion rendered the older eggs inviable, whereas the fresher ones developed and hatched successfully. At the time of immersion, the more developed eggs would be expected to have greater gas exchange needs. Secondly, some eggs in Brown & Alcala's experiment remained viable after eleven days of wetting. Gas exchange through the thin layer of water on the egg surface would be relatively unimpeded in this experimental design. Hence, successful long distance rafting may require the eggs to be above water for some of the time, or at least exposed to air fairly frequently. As the hollow side of a tree trunk is more likely to float uppermost, this may often happen. Indeed, as cooler sea temperatures may reduce the respiration rate of the embryos, and the surface sea water is likely to be more oxygenated than that in this experiment, the likelihood of survival may be higher.

Brown & Alcala also tested the eggs of the skink species *Dasia smaragdina philippinica*, and found that none of their parchment shelled eggs remained viable after relatively short periods of exposure to sea water. Hence the characteristics of gecko eggs, including the resistant shell, long incubation times, communal oviposition and tolerance of the eggs to sea water, strongly suggest that rafting of gecko eggs may contribute to the wide distribution of the family on oceanic islands.

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ASPECTS OF THE ECOLOGY OF *TESTUDO HERMANNI* IN SOUTHERN YUGOSLAVIA

R. MEEK

8 *Mountfield Road, Waterloo, Huddersfield, England*

(*Received* 1 *March* 1984)

SUMMARY

In 1978 a preliminary study was made on a population of Hermann's tortoise *Testudo hermanni* in southern Yugoslavia (Meek & Inskeep, 1981). New observations have now been made on this population and information mainly concerning size and age frequencies, sex ratios, population densities, food plants, injuries and shell abnormalities and morphometrics have been gathered. Female *T. hermanni* grow significantly longer (carapace length) and heavier than males. Male tortoises in 1978 were significantly heavier than those sampled in 1983. Males outnumber females by 2:1; adults outnumber immatures by 9.2:1. Eighty-six percent of tortoises were 14 years or more in age. Mathematical models of tortoises age predict that large tortoises (⩾200 mm) may exceed 40 years in field populations. Survivorship until 19 years was 38.3% and mean annual recruitment estimated at 3.4%.The Schnabel formula has been used to calculate population densities and indicates a mean density of 39.2 ha^{-1}. The principal food plants included the families Leguminosae (33.3%) and Ranunculaceae (25%), the latter and Aracaea comprised species containing toxic alkaloids (41.6%). In a sample of 82 tortoises, 14.6% showed abnormalities to the shell scutelation and physical injuries. Allometric equations have been produced from the measurements of shell dimensions and body mass. It has been found that most of the allometric exponents are close to the 0.33 required for geometric similarity, except those relating supracaudal width and plastron length to body mass in males. This can be explained by smaller growth increments of male plastrons and larger increments in male supracaudal scute width. Plastron length has also been related to carapace length; the relationship is isometric and the regression equations for males and females are significantly different.

INTRODUCTION

Hermann's tortoise (*Testudo hermanni*) constitutes one of the three species of European terrestrial chelonians (Arnold, Burton & Ovenden, 1978). In the form of two races, *T. h. hermanni* and *T. h. robertmertensi*, it is found in a variety of habitats principally in the warmer southern regions of Europe. Until recently, however, field data on the species was virtually non-existent, despite its large scale collection for the pet and food trades (Lambert, 1980). The first field study of *T. hermanni* was made by Chelazzi & Francisci (1979) who investigated movement patterns and homing behaviour of an Italian population of *T. h. robertmertensi*. This was followed by two, almost simultaneous studies of populations of *T. h. hermanni*, in Greece (Stubbs, Hailey, Tyler & Pulford, 1981) involving large scale ecological work, and in Yugoslavia (Meek & Inskeep, 1981) where the general field biology of the species was investigated. More recently (Meek, 1984) thermoregulatory behaviour in this Yugoslavian population has been studied adding to the earlier laboratory studies of *T. hermanni* thermoregulation by Cherchi (1956, 1960). Auffenberg & Weaver (1969) when working with the North American tortoise *Gopherus berlandieri* drew attention to the possibility that demographic information generated for one tortoise population may not always be applicable to others, even when these may be in close proximity. Comparative ecological information on tortoise populations is therefore not only of general interest but is also a necessary practical conservation tool. This paper is a contribution to the field studies of *T. hermanni* and examines certain aspects of the species ecology in southern Yugoslavia.

METHODS AND MATERIALS

Field work was initially carried out in 1978 on a population of *T. hermanni* from Montenegro in southern Yugoslavia, and the preliminary results of this work have been published (Meek & Inskeep, 1981). Additional data were gathered in May 1983 on this population. Further information, mainly growth ring counts and carapace lengths, were gathered from tortoises imported into Britain in May–June 1983, which were reported to have been collected in the southern Yugoslavian province of Macedonia.

HABITAT AND FOOD PLANTS

Samples of the principal shade and food plants of the habitat were collected. Food plants were those actually observed being consumed by the tortoises. The specimens were pressed and later identified at the Department of Plant Sciences, University of Leeds.

BODY MEASUREMENTS

Carapace length. A straight line between the leading edges on the supracaudal and nuchal scutes.

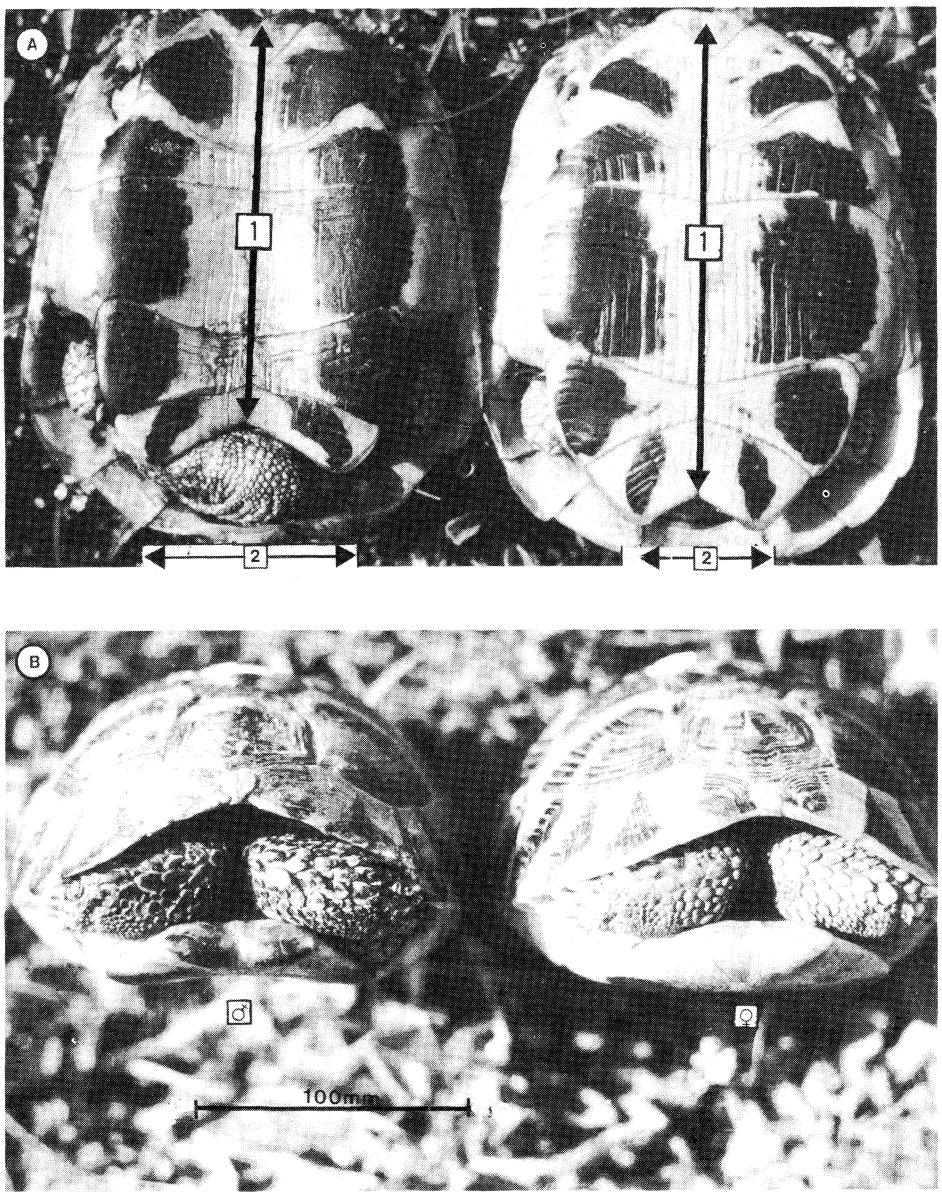

FIG. 1. Measurements of plastron length (1) and supracaudal scute width (2) in adult male (left) and female *T. hermanni*. Figure 1B shows the more concave plastron of males; Figure 1A the larger more prominent tail of male tortoises.

Plastron length. A straight line from the leading edge on the gular to the notch on the anal scute. This measurement is shown in Fig. 1.

4th vertebral scute. A straight line on the width.

Supracaudal scute. A straight line on the leading points on the width. This measurement is shown in Fig. 1.

Body mass. This was determined by weighing in a cloth bag suspended from a spring balance.

Assessing Sex

Male and female tortoises were distinguished by the concave plastron in males and shorter tails in females; this is shown in Fig. 1. Tortoises of less than 110 mm carapace length could not be sexed confidently.

Physical Condition

Records of physical injuries and shell damage were made in both written and photographic form. Only major injuries were noted, minor shell damages (which were quite common) were ignored.

Population Density Estimates

A five day mark-release-recapture survey was carried out to estimate population densities. Tortoises were collected and given codes specifying date and time of capture. The coding was painted on the carapace of each animal using Tippex fluid, which was found suitable for a short term survey. To estimate population densities a variation on the Lincoln Index, the Schnabel method (1938) has been used. This method

allows calculation on a daily basis, smoothes the data by accumulation and renders the estimates uniform (Davis, 1963). Graham (1979) has demonstrated the mechanics of this method, discussing its problems and the various basic assumptions that should be taken into account; these are believed to have been satisfied for this survey.

Statistical Analysis

From the measurements of the shell dimensions, the number of growth annuli on the costal scutes and body mass allometric equations have been obtained by the method of least squares regression after transforming the data into logarithmic form (Bailey, 1959). Since body mass represents the whole animal this measurement has been treated as the independent variable in the morphometric analysis; growth annuli have been treated as dependent on carapace length or 4th vertebral scute width. The method of Kermack & Haldane (1950), which makes no distinction between independent and dependent variables, would produce only slightly different equations when the correlation coefficients are 0.92 or higher. The exponents would be found by calculating $(1/r)b$, where r is the correlation coefficient and b the exponent given here (Alexander *et al.*, 1979). Using non-transformed data the least squares method has been used to relate carapace length to plastron length. The t distribution has been used to assign 95% confidence intervals to the exponents in the allometric equations and to factor m in the regression equations. Tests for significant differences between equations have been made by analysis of variance.

RESULTS

Habitat

In the study area (typical Mediterranean mixed scrub), *T. hermanni* was found in association with *Clematis viticella*, *Rubus fruticosus*, *Cornus sanguinea*, *Berberis vulgaris*, and *Paliurus spina-christi*, which were utilized as shade plants. Tortoises were also found moving through extensive growth of bracken, *Pteridium aquilinum*, when these bordered major shade plants.

A preliminary survey of the herpetofauna recorded on the study area has been reported (Meek & Inskeep, 1981). Further searches revealed *Triturus vulgaris dalmatica* and *Rana ridibunda*, and one reptile *Mauremys caspica rivulata*. Few truly wild mammals have been observed, but in recent years, clearly as a result of a large scale building programme on the study area and subsequent human population influx, there has been an increase in the number of feral cats and domestic goats.

Population Structure

Size (carapace length) and body mass frequency distributions of tortoises in 1978 and 1983 are shown in Fig. 2. In both sampling periods the frequency distributions were skewed towards larger animals, the majority of animals had carapace lengths above 120 mm (1978 = 74%; 1983 = 76%) and body masses greater than 500 g (1978 = 68%, 1983 = 79%). Female *T. hermanni* attained longer carapace lengths (1978 = 174 mm, $\bar{x}$ = 145 mm, 1983 = 170 mm, $\bar{x}$ = 147 mm) than males (1978 = 160 mm, $\bar{x}$ = 124 mm; 1983 = 143 mm, $\bar{x}$ = 126 mm). The differences were significant; 1978, $F(1, 34) = 4.25$, $P < 0.05$; 1983, $F(1, 72) = 72.9$, $P < 0.001$. Female tortoises also attained greater body mass (1978 = 1160 g, $\bar{x}$ = 861 g; 1983 = 1410 g, $\bar{x}$ = 989 g) than males (1978 = 1000 g, $\bar{x}$ = 637 g; 1983 = 740 g, $\bar{x}$ = 609 g). The differences were significant; 1978 $F(1, 34) = 10.43$, $P < 0.005$; 1983 $F(1, 72) = 108.5$, $P < 0.001$. There was no significant difference between the carapace lengths of females measured in 1978 against those in 1983, $F(1, 37) = 0.21$, $P > 0.05$ or between their masses, $F(1, 37) = 2.77$, $P > 0.05$. There was also no significant difference between the carapace lengths of males, $F(1, 69) = 0.82$, $P > 0.05$, but the 1978 males were significantly heavier $F(1, 69) = 10.5$, $P < 0.005$.

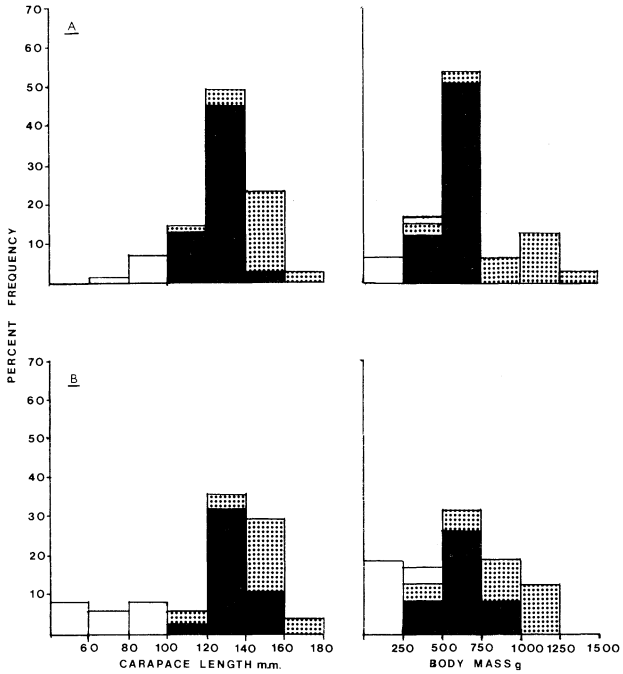

FIG. 2. Length and body mass frequencies of *T. hermanni* in 1983 (A) and 1978 (B). The 1983 carapace length and body mass histograms are based on 82 tortoises, 1978 carapace lengths $n = 47$, 1978 body mass $n = 43$. Open histograms represent juveniles, solid areas males and stipple areas females.

Sex Ratios

If sex ratios are based on tortoises caught on a daily basis, this gives an average daily ratio of 1.8:1 in favour of males, varying from 1.1:1 to 2.6:1. Based on the total number of identified tortoises measured on the study area (82) a male/female ratio of 2:1 was recorded and an adult-juvenile ratio of 9.2:1.

Age Structure

Age frequencies of *T. hermanni* are shown in Fig. 3. Figure 3b is based on estimated ages by use of

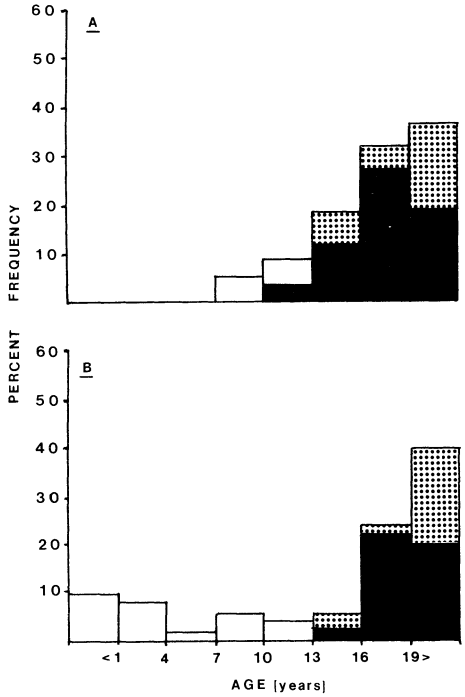

FIG. 3. Age frequency histograms of *T. hermanni* in 1983 (A), $n = 82$ and 1978 (B), $n = 47$. Open histograms represent juveniles, solid areas males and stippled areas females.

TABLE I. Allometric equations of the form $y = ax^b$ relating the number of growth annuli on the costal scutes y with either carapace length or 4th vertebral scute width x in mm by the constants a and b. The equations for males and females have been calculated by including the data from juveniles. These equations are derived from field and recently imported *T. hermanni*

Eqn. number	Independent variable x	a	b	95% confidence interval on b	n
(1)	Carapace length (males)	0.0003	2.29	0.53	72
(2)	Carapace length (females)	0.0005	2.12	0.62	46
(3)	Carapace length (pooled)	0.0005	2.15	0.42	98
(4)	4th vertebral scute (males)	0.005	2.37	0.57	61
(5)	4th vertebral scute (females)	0.009	2.20	0.66	40
(6)	4th vertebral scute (pooled)	0.008	2.25	0.44	91

equations (1) and (2) in Table I. The age distributions were skewed towards older animals in both periods with 40% and 36.6% in the 1978 and 1983 samples respectively of 19+ years. There was an approximate equal distribution of males and females in the 19+ years age class during both periods but males predominate between 16–19 years. The growth annuli counts of males averaged 19.2 (S.D. = 3.6), females 21 (S.D. = 8.8). The differences between the sexes were not significant, $F(1, 54) = 1.03$, $P > 0.05$. The average growth annuli count for the total sample ($n = 82$) including juveniles was 18.3 (S.D. = 5.6). Juveniles and immatures were well represented in the 1978 sample but mostly absent in the 1983 data. It is not known whether this is a natural situation or a function of sampling error; smaller tortoises being more difficult to locate particularly in dense vegetation.

AGE MODELS

The number of growth annuli on the costal scutes are generally correlated with carapace length ($r = 0.96$) and with 4th vertebral scute width ($r = 0.95$). For carapace length the correlation is slightly higher for males ($r = 0.98$) than females ($r = 0.97$) and for 4th vertebral scute width also higher for males ($r = 0.96$) than females ($r = 0.95$). The relationships can be described by the allometric equation

$$y = ax^b$$

where the number of growth annuli y is related to the length of the carapace or width of the 4th vertebral scute x by the constants a and b. Figure 4 shows the data plotted on logarithmic coordinates; Table I the equations derived from the data. The results indicate that males grow more slowly than females since (for example), equations (1) or (4) for males will at any given measurement predict higher numbers of annuli than equations (2) or (5). However, there is no significant difference ($P > 0.05$) between equations (1)

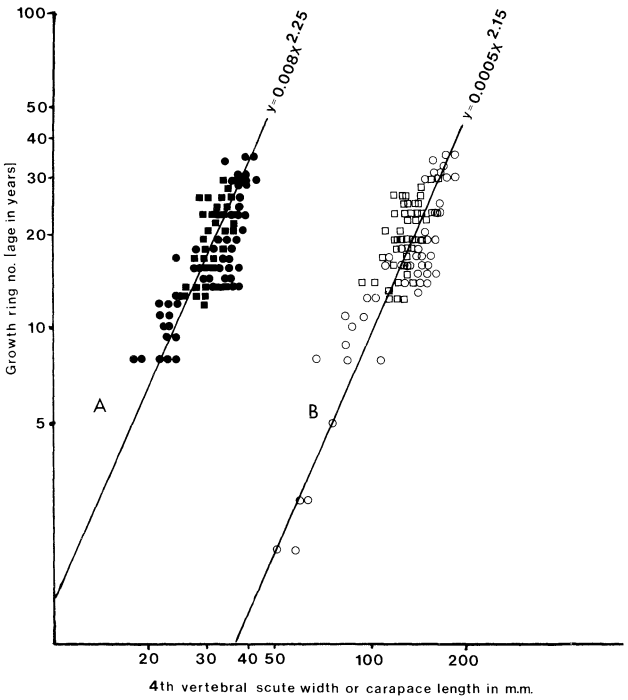

FIG. 4. A graph on logarithmic coordinates of the number of growth annuli plotted against 4th vertebral scute width and carapace length. The lines taken through the data represent equations (3) and (6) given in Table I. When 4th vertebral scute width *vs.* annuli males = ■, females and juveniles = ●. When carapace length *vs.* annuli, males = □, females and juveniles = ○. These data are based on wild tortoises and recently imported animals.

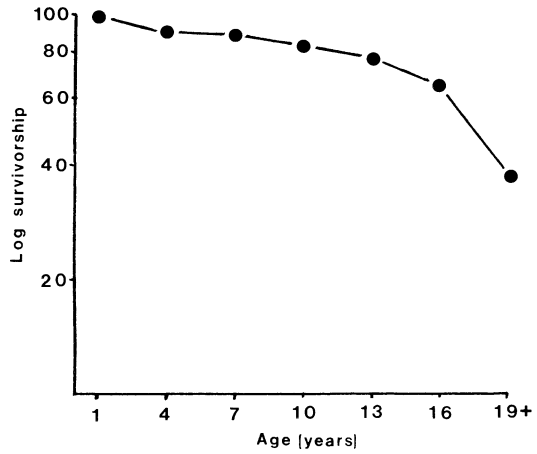

FIG. 5. Survivorship of Yugoslavian *T. hermanni* field populations until 19+ years. This graph is based on the pooled data from the 1978 and 1983 field samples ($n = 129$).

and (2) or between equations (4) and (5). The lines taken through the data in Fig. 4 are derived from equations (3) and (6). These general equations predict that large tortoises (*i.e.* ⩾200 mm carapace length) may exceed 40 years in field populations.

SURVIVORSHIP

Age determination in *T. hermanni* has enabled the construction of a survivorship curve (Fig. 5). This curve has been calculated by taking, as a percent of the total population sample (1978 and 1983, $n = 129$), the number of tortoises present at the beginning of each age class (Deevey, 1947). Survivorship until 19 years is 38.3% and the mean annual recruitment based on the pooled 1978–1983 age data estimated at 3.4%.

POPULATION DENSITIES

Population densities have been calculated using the Schnabel formula,

$$\hat{P} = \frac{\sum [m(u + r)]}{\sum r}$$

where the population density $\hat{P}$ is estimated from the number of tortoises captured, marked and released m, the number of marked individuals recaptured each day r and the number of unmarked animals captured each day u. The summations are over the number of days, thus m and $\sum r$ become progressively larger. Table II shows the results and the arithmetic involved in the survey as it was applied to an area of 0.8 ha. Overton (1971) discusses the methods of averaging the results. However, in this short term survey the estimates become uniform only after the second day of sampling. Extrapolation of the average of the third, fourth and fifth days results for 0.8 ha gives a population density estimate of 39.2 ha^{-1}.

When mass is unknown, biomass density can be estimated from,

$$\hat{b} = (0.0013\bar{U}^{2.7})N \qquad (7)$$

where the biomass density $\hat{b}$ in g is determined from the mean carapace length of the population $\bar{U}$ in mm and the population density N. This gives a biomass density estimate in *T. hermanni* of 24 183 g ha^{-1}.

TABLE II. Estimates of population densities $\hat{P}$, of *T. hermanni* in Yugoslavia using the Schnabel method. The results are based on a survey in an area of 0.8 ha and the table shows the results and the arithmetic involved during the five day survey. The Schnabel equation is given in the text

Day	$u + r$	m	$\Sigma [m(u + r)]$	r	Σr	$\hat{P}$
1	21	—	—	—	—	—
2	26	21	546	6	6	91
3	18	30	540	9	15	36
4	18	39	702	9	24	29.2
5	23	48	1104	14	38	29

FOOD PLANTS

A total of twelve plant specimens of several species were collected from feeding *T. hermanni* (Table III). In agreement with the 1978 sample (Meek & Inskeep, 1981) the family Leguminosae (33.3%) persists with the family Ranunculaceae (25%) as the principal food plant groups of *T. hermanni* and both samples combined formed 58.3% of all the food plants that were collected. The Graminea are also again represented (8.3%) but two new families, the Araceae and Rubiaceae which were not recorded in the earlier survey formed 16.6% and 8.3% of collected plants respectively. One additional specimen was not identified. Also indicated in Table III is the presence of toxic alkaloids in five (41.6%) of the food plants of *T. hermanni*.

ABNORMALITIES AND INJURIES

In a total of 82 tortoises examined, twelve (14.6%) showed abnormalities in scutelation. Two of these (2.4%) appeared to be congenital peculiarities arising during growth. Of those that appeared to have resulted from injuries almost all (12.2%) involved the carapace,

TABLE III. A list of plant specimens on which *T. hermanni* were observed feeding. Although no definite identification can be made to the species from the family Araceae, the probable species are shown in parenthesis. The presence of alkaloids in plant species are also indicated.

Family	Species	Alkaloids present
Leguminosae	*Medicago polymorpha*	—
Leguminosae	*Vicia sativa*	—
Leguminosae	*Vicia sepium*	—
Leguminosae	*Trifolium campestre*	—
Ranunculaceae	*Clematis viticella*	Yes
Ranunculaceae	*Clematis* sp.	Yes
Ranunculaceae	*Ranunculus acris*	Yes
Araceae	*Arum* sp. (*italicum, maculatum* or *orientalis*)	Yes
Araceae	*Arum* sp. (*italicum, maculatum* or *orientalis*)	Yes
Gramineae	*Dactylis glomerata*	—
Rubiaceae	*Galium* sp.	—

TABLE IV. Allometric equations of the form $y = ax^b$ relating selected dimensions of the shell y in mm to body mass x in g by the constants a and b. The t distribution has been used to assign 95% confidence limits to the exponent b. The r value is the correlation coefficient, n the number of measurements on which the equations are based.

Eqn. number	Dependent variable y	a	b	Confidence limits on b	r	n
(8)	Carapace length (males and juveniles)	11.92	0.37	0.06	0.81	78
(9)	Carapace length (females and juveniles)	14.31	0.35	0.10	0.90	57
(10)	Carapace length (pooled)	14.20	0.34	0.06	0.96	126
(11)	Plastron length (males)	26.20	0.22	0.07	0.71	32
(12)	Plastron length (females)	13.26	0.33	0.04	0.95	19
(13)	Plastron length (pooled)	13.50	0.32	0.08	0.96	59
(14)	4th vertebral scute (males and juveniles)	4.07	0.32	0.08	0.91	63
(15)	4th vertebral scute (females and Juveniles)	4.33	0.30	0.11	0.97	39
(16)	4th vertebral scute (pooled)	5.84	0.27	0.05	0.92	91
(17)	Supracaudal width (males and juveniles)	3.33	0.41	0.13	0.94	41
(18)	Supracaudal width (females and juveniles)	4.36	0.35	0.16	0.95	18
(19)	Supracaudal width (pooled)	6.56	0.30	0.07	0.81	57

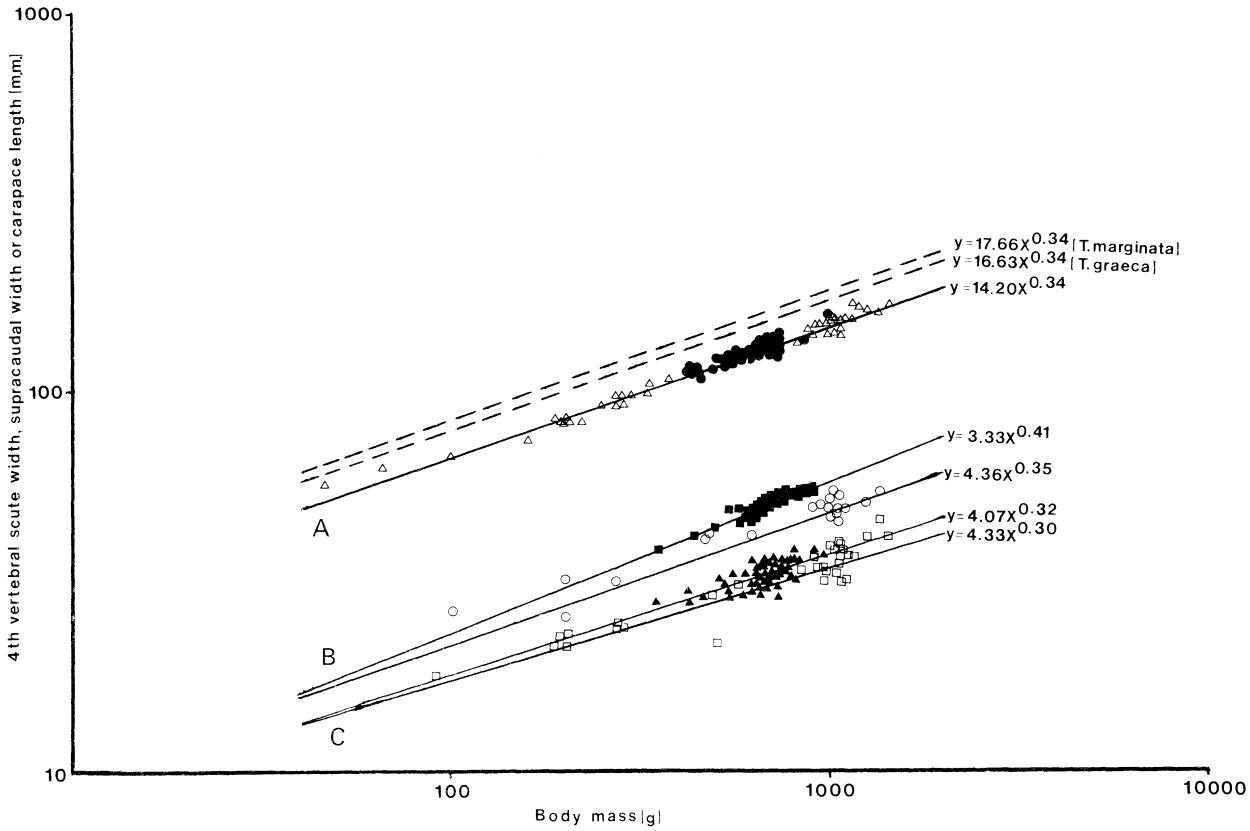

FIG. 6. A graph on logarithmic coordinates of body mass plotted against 4th vertebral scute width, supracaudal scute width and carapace length. Lines taken through the data represent allometric equations given in Table IV. For comparative purposes lines predicted by the allometric equations for *Testudo graeca* calculated by Lambert (1982) and *T. marginata* based on Hines (1982) data are shown with that from the general equation (10) for *T. hermanni* calculated in this study. Details of the symbols are as follows: When body mass *vs.* 4th vetebral scute width: males = ▲, females and juveniles = □; when body mass *vs.* supracaudal width: males = ■, females and juveniles = ○; when body mass *vs.* carapace length; males = ●, females and juveniles = △.

although two tortoises showed damage to both the carapace and the plastron. One animal had plastron damage only. Another tortoise had a break in the lower jaw at the anterior junction of the dentary bones to the effect that both sides of the lower jaw worked independently. However, this disability did not appear to affect its ability to feed since it was observed feeding successfully on three occasions.

MORPHOMETRICS

Allometric equations describing the relationship between the various shell dimensions and body mass in *T. hermanni* are shown in Table IV. The equations are of the same basic form as those relating growth annuli with shell measurements except that here a shell character y in mm is related to the body mass x in g by

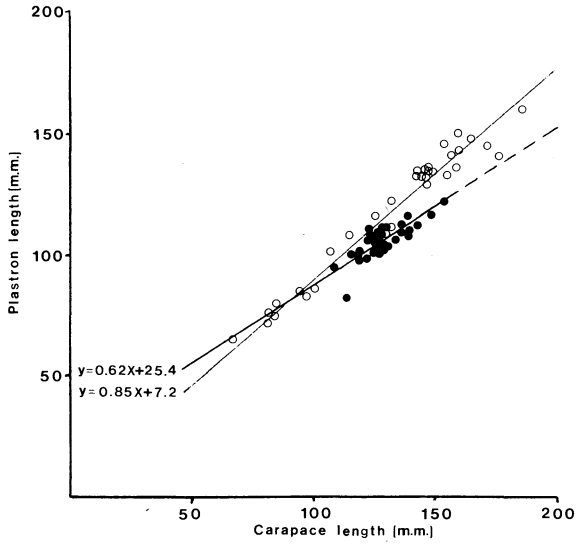

FIG. 7. A graph of carapace length plotted against plastron length. The lines represent equations (20) and (21) given in the text. The symbols are males = ●, females and juveniles = ○.

the intercept a and exponent b. The separate male and female equations have generally been calculated incorporating the data from juveniles since this method to some extent alleviates the bias in size distribution of field examined/collected tortoises and the effect that such a bias may have on the equations, correlation coefficients and confidence intervals, particularly when n is low.

Most of the allometric exponents agree well with the 0.33 required for geometric similarity. Significantly different exponents, however, have been found for male plastron length ($b = 0.22$; $P < 0.01$) and supracaudal width ($b = 0.41$; $P < 0.01$) indicating important sexual dimorphic characters (*i.e.* shorter plastrons and greater supracaudal scute width in males) in the shell structure of *T. hermanni*. These data are shown in Fig. 6.

Pooling the data improves the confidence intervals in equations (16) and (19) and may also produce a change of exponent to one that may not lie between the exponents of the male and female equations [equations (10), (16) and (19)]. In such cases the exponents are reduced, which indicates that tortoises become relatively heavier as they grow.

There is a high correlation and an isometric relationship when plastron length is plotted against carapace length in both males ($r = 0.95$) and females ($r = 0.98$). The regression equations derived from these measurements have been calculated for each male and female equation using the data from juveniles and have the form,

$$y = mx + b$$

where the plastron length y in mm is related to the carapace length x in mm by the slope m and the y intercept b. This supports the general trend for longer plastrons in females since,

$$y = 0.62 \pm 0.20x + 25.4 \text{ (males, } n = 41) \quad (20)$$

$$y = 0.85 \pm 0.32x + 7.2 \text{ (females, } n = 30) \quad (21)$$

with the slopes significantly different ($P < 0.001$). Pooling the data also gives a high correlation ($r = 0.94$) and reduced the confidence interval thus,

$$y = 0.84 \pm 0.21x + 3.07 \ (n = 64) \quad (22)$$

Figure 7 is a graph of plastron length plotted against carapace length with lines derived from equations (20) and (21) taken through the data. The broken line extrapolates the equation for males (20) to the general maximum carapace length for *T. hermanni* (Arnold *et al.*, 1978).

DISCUSSION

Of the ecological factors which govern the stability of tortoise populations one of the most critical is undisturbed habitat. The movement into the study area of parts of the local human population from areas destroyed by the earthquakes of 1979–80 and the inevitable land changes that have subsequently taken place has notably reduced the numbers of tortoises generally encountered on the study site. These developments have mostly had the effect of restricting the remaining tortoise population to small isolated pocket communities separated by extensive housing areas. The introduction of goats into the remaining habitat is an additional factor that may ultimately influence the stability of the remaining population since these have already been shown to affect the survival of Galapagos giant tortoises *Geochelone elephantopus* by competing with the tortoises for food and trampling the nest sites (MacFarland, Villa & Toro, 1974). Goats may also compete with certain other tortoise species for food plants (Morafka, 1982). However, feral cats could have a more immediate effect by preying on the eggs and hatchling tortoises.

Skewed size and age frequency distributions towards large or older individuals have been found in a number of populations of terrestrial chelonians. The size and age frequencies of Yugoslavian *T. hermanni* closely approximate those recorded for Greek *T. hermanni* (Stubbs *et al.*, 1981) and North African and Turkish populations of *T. graeca* (Lambert, 1982), although the tortoises recorded in this study did not attain the size of the animals measured in these earlier works. A bias towards large or adult tortoises has been found in North American *Gopherus agassizi* (Reyes Osorio & Bury, 1982) and *G. berlandieri* (Rose & Judd, 1982) and also in giant tortoises *Geochelone gigantea* (Grubb, 1971; Bourn & Coe, 1978). Information on the age structures of Mediterranean tortoises has revealed low recruitment rates varying from 1–5% (Stubbs *et al.*, 1981; Lambert, 1982 and this paper) and late maturity (13–14 years, Castanet & Cheylan, 1979). These data indicate that Mediterranean tortoises operate at the K end of the $r - K$ continuum maintaining stable populations.

Males predominate in *T. hermanni* field populations (Stubbs *et al.*, 1981 and this study) although not necessarily in all Mediterranean *Testudo* where females may be more numerous (Lambert, 1982). Mortality in female *T. hermanni* may differ from males since females incur greater energy costs in egg production.

This would not of course explain why greater numbers of females are sometimes found in *T. graeca* populations (Lambert, 1982). Sex in *Testudo* is known to be temperature dependent (Bull, 1980) where high incubation temperatures of the eggs produce females and lower temperatures males. Lambert (1982) has drawn attention to environmental sex determination as a possible explanation for differences between the sex ratios of Turkish and North African populations of *T. graeca*.

A method of assessing the age of tortoises in field studies when growth annuli are worn smooth is of particular importance in order that the population dynamics can be more fully understood. The mathematical models of age assessment presented in this paper are dependent on two factors. The first is that the number of growth annuli on the carapace does indeed reflect a tortoise's age and secondly that age can be correlated with carapace length or 4th vertebral scute width. These conditions are satisfied by the high correlations for the data and also by the work of Castanet & Cheylan (1979) who examined osseous growth marks of the long bones of *T. hermanni* and *T. graeca* and found that these were in good agreement with shell annuli at least until 19 years. The equations relating growth annuli to carapace length have better confidence intervals and slightly higher correlation coefficients than those relating growth annuli to 4th vertebral scute width. Nevertheless, there is little disagreement in the predictions made by the corresponding sets of equations. However, it should be noted that Kirsche (1979) found differences in age related growth in captive bred *T. hermanni* but Bourn & Coe (1978) found a valid relationship between age and size in a population of *Geochelone gigantea*. With their equations, Bourn & Coe estimated that *Geochelone* may attain between 50–70 years which considerably exceeds the 40 years predicted in this paper for the much smaller *T. hermanni*.

Growth studies of Mediterranean tortoises include those of *T. hermanni* (Meek & Inskeep, 1981; Stubbs *et al.*, 1981), *T. graeca* (Lambert, 1982) and captive *T. hermanni* and *T. graeca* (Meek, 1982). Hine (1982) gathered information on carapace length and body mass in wild *Testudo marginata* in Greece but did not quantify his measurements. However, from his data it is calculated that

$$y = 17.66x^{0.34 \pm 0.14} (r = 0.98, n = 24) \qquad (23)$$

The exponent is in agreement with those for field *T. hermanni* (this paper) and *T. graeca* (Lambert, 1982), thus identical exponents, close to a geometric similarity model have been found for all three species of European *Testudo*. However, the equations have different intercepts (factor a). Figure 6 shows lines predicted by the equation for *T. graeca* calculated by Lambert (1982) and that calculated for *T. marginata* for comparison with the equation for *T. hermanni* demonstrating the effects of the different intercepts on the elevation of the lines predicted by the equations. This shows that Yugoslavian *T. hermanni* appear to be relatively heavier than either *T. graeca* or *T. marginata* which is possibly a function of the quality or quantity of food available in each species habitat, rather than some inherent difference in mass status. Yugoslavian *T. hermanni* also appear to be heavier than specimens from Greece (Stubbs *et al.*, 1981; b = 0.35, a = 16.00 estimated in Meek, 1982) but it is interesting that rather different exponents have been calculated for captive *T. hermanni* (b = 0.38) and *T. graeca* (b = 0.30) by Meek (1982). This might be explained by the differences in diet and activity levels between captive and free-living tortoises.

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ASPECTS OF THE POPULATION AGE-STRUCTURE OF THE COMMON TOAD (*BUFO BUFO*) AT LLANDRINDOD WELLS LAKE, MID-WALES

S. P. GITTINS, R. I. KENNEDY AND R. WILLIAMS

Department of Applied Biology, University of Wales Institute of Science and Technology, PO Box 13, Cardiff CF1 3XF

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SUMMARY

1. The toad population at Llandrindod Wells Lake, measured over a four-year period for females and a two-year period for males, showed an apparently stable age distribution.
2. Female toads were estimated to have matured at between 3 and 6 years of age, males at between 2 and 3 years.
3. The oldest individual in the sample was 9 years; few individuals appear to have lived for more than 6 years.
4. There was no significant relationship between body length and age in mature toads.
5. Bone deposition declined markedly with age.

INTRODUCTION

Age determination of amphibians has been achieved by counting the growth-rings in cross-sections of bone, corresponding to annual periods of summer growth (Smerna, 1972; Hagstrom, 1977; Hemelaar & van Gelder, 1980; Gittins, Steeds & Williams, 1982). In this study the method has been used to investigate the stability of the age-distribution of the common toad (*Bufo bufo*) visiting a lake in mid-Wales during the breeding season.

METHODS

The toads used in this study were collected as casualties from the road surrounding Llandrindod Wells Lake, Powys (SO 063605). The animals were obtained during the breeding migration to the Lake and are assumed to be sexually mature. Samples of female toads were available for 1978 ($n = 12$), 1981 ($n = 7 + 20$ from a previous study, Gittins, Steeds & Williams 1982), 1982 ($n = 20$), with a combined sample for 1979/80 ($n = 16$). For males, only the samples for 1981 ($n = 31$ from Gittins, Steeds & Williams 1982) and 1982 ($n = 65$) have been analysed. The toads were stored, deep frozen, and before processing thawed and kept in 70% alcohol at room temperature. Each specimen was given a coded label and its dorsal snout-urostyle length measured to the nearest mm.

The method used for ageing the toads followed the procedure of Gittins, Steeds & Williams (1982) which is based on that of Hemelaar & van Gelder (1980). Growth-rings corresponding to periods of increased growth have been found in cross-sections of the bones of various amphibians, and in populations forced to hibernate these growth-rings correspond to annual periods of summer growth (Hagstrom, 1977). Growth-rings consist of relatively broad layers of periosteal bone, separated by narrow "resting lines" which represent the hibernation period (see Gittins, Steeds & Williams, 1982 for a photograph showing the growth rings). One complete year's growth is represented by one growth-ring and one resting line (Hemelaar & van Gelder, 1980). As the Lake population was observed in the spring, before growth had re-commenced, the outer edge of each section was counted as a resting line.

Hemelaar (1981) showed that resorption of the first year's growth occurred in about 6% of the total sample, with the remaining 94% displaying all their year-rings. During the four years prior to 1982 some individuals were given year marks by toe-clipping, allowing the youngest possible age for certain recaptured toads to be determined. Thirteen individuals from the sample had been toe-clipped and of these, six had been under-aged by one year, and young individuals were as likely to be under-aged as older individuals.

Bone deposition in each year of life was investigated by measuring the thickness of each year-ring using a microscope with an eye-piece graticule at a magnification of 100×. These measurements were converted into percentage relative growth-increment per year, to compensate for sections cut from different regions of the bone and at different tangents across the bone. To allow comparison between ages, these percentages were recalculated taking a specific age (the oldest possible with a large n) as a standard, and recalculating the other years according to cumulative growth for each year. For example, for females the five-year-old group was used as the standard, and the 100% of the diameter of three-year-old females recalculated at 71%, the cumulative growth after three years of five-year-old females.

All statistical tests were carried out by computer using the Minitab Statistical Package (Ryan, Joiner & Ryan, 1981).

RESULTS

The population age-structures of males and females for 1982 are shown in Fig. 1. These distributions were compared with the distributions for the same population in 1981 (Gittins, Steeds & Williams, 1982) using a Kolmogorov–Smernov two-sample test, showing no

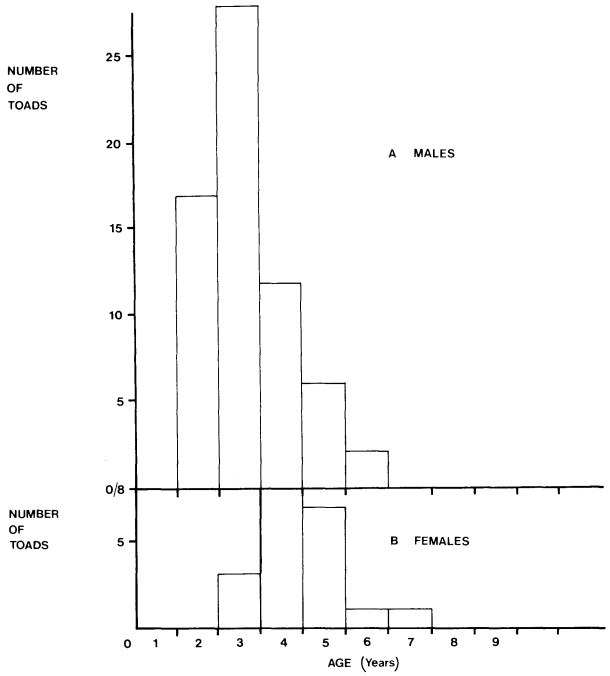

FIG 1. Population age-structure for male and female toads in 1982. Males $n = 65$; females $n = 20$.

significant differences between years for either males or females (males $D = 0.14$, $P > 0.05$; females $D = 0.20$, $P > 0.05$). Data were pooled for females collected in 1978, 1979 and 1980 and the resulting age-distribution compared with the 1981 and 1982 data; no significant difference in age-structure was observed (1982 $D = 0.15$, $P > 0.05$; 1981 $D = 0.09$, $P > 0.05$) As the population age-structure indicated stability, the results of this study were combined with those of Gittins, Steeds & Williams (1982); the resulting distributions were presented in Fig. 2.

There is an increase in numbers between the youngest age of breeding and the following year suggesting that not all individuals mature at the youngest possible age. Once maturity is reached there should be a decline in numbers that relates to the annual mortality rate, which, in this population is in the region of 40–50% (Gittins, 1983). Males attained maturity at two or three years of age whereas females show a greater variability with maturity being attained between 3 to 6 years of age. The maximum age in the sample of females was 9 years, and for males 6 years, and only a very small proportion of toads lived beyond 6 years in the wild.

There were no significant differences between the average length of males or females in 1982 (males 57.5 mm, females 71.0 mm) and 1981 (males 57.3; females 69.5) ($P > 0.05$). A regression of length against age showed no significant relationship for either male or female toads (males 1982 $n = 65$, $r = 0.008$, $F = 0.507$, $P > 0.05$ and the combined sample for females $n = 55$, $r = 0.003$, $F = 0.134$, $P > 0.05$), suggesting that linear growth is insignificant once sexual maturity has been attained.

The relative growth increments are shown in Table 1 and Fig. 3. It is the shape of the graph that is important here rather than specific values for the increment. From Fig. 3 it can be seen that the largest increments occur at the younger ages, and these decrease the year before sexual maturity is attained. After maturation bone diameter continues to increase but at a declining rate.

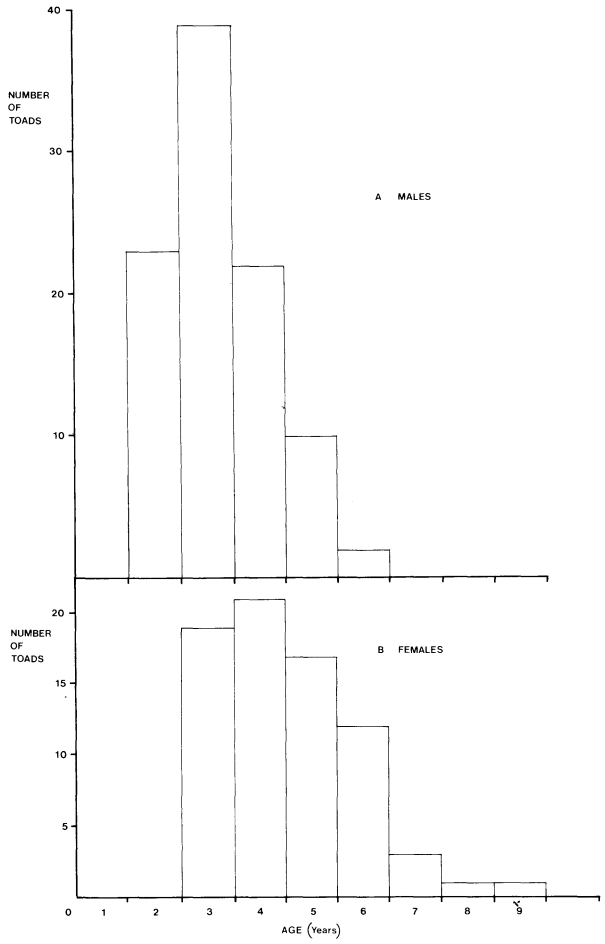

FIG. 2. Combined population age-distribution. Males 1981–82, $n = 96$; females 1978–82, $n = 75$.

TABLE I. Relative growth increments in bone

(a) Males 1982 based on 4-year-olds

		Age of increment					
Age	N	1	2	3	4	5	6
2	13	37	25				
3	25	34	29	19			
4	11	29	32	20	18		
5	6	42	22	20	16	17	
6	1	24	24	30	21	24	12
	56	33	26	22	18	21	12

(b) Females 1982 based on 5-year-olds

		Age of increment						
Age	N	1	2	3	4	5	6	7
3	3	23	27	19				
4	8	29	25	17	14			
5	7	21	27	23	17	12		
6	1	29	26	24	12	9	8	
7	1	19	19	17	22	22	7	7
	20	24	25	20	16	14	8	7

DISCUSSION

Under constant conditions many species display a stable age-distribution. That found at the Lake might be

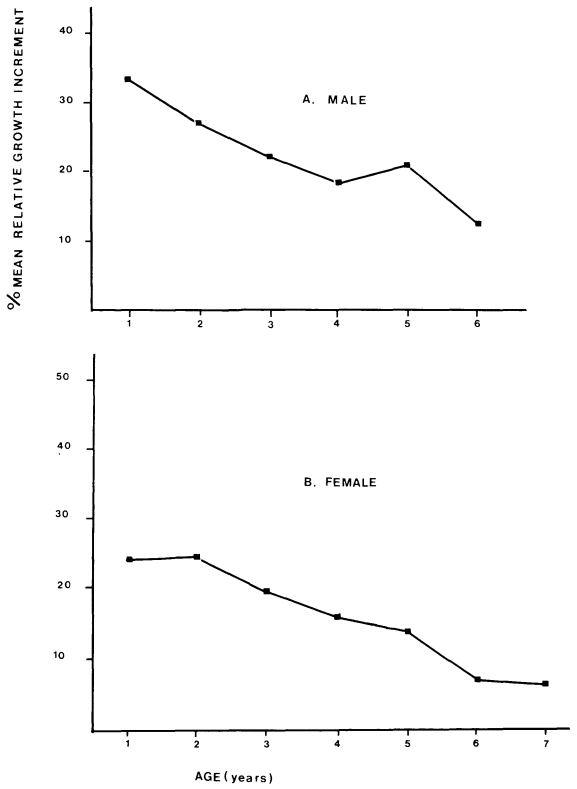

FIG. 3. Relative growth increment in bone. Values for growth increment were calculated as described in the text.

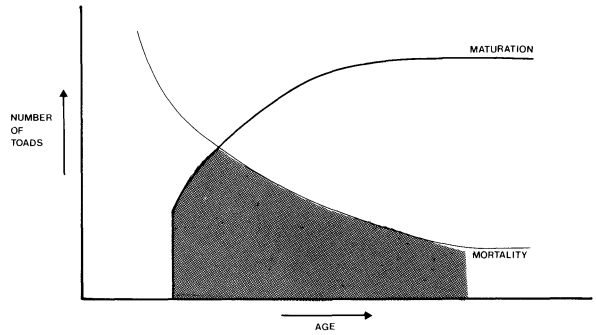

FIG. 4. Graph showing the interaction of maturation and mortality on population age-structure.

expected since population size has remained remarkably constant over the five-year study period (Gittins, 1983). Males attained sexual maturity between the ages of two and three, and females between three and six years, showing great similarity with the toad population studied in Holland (Hemelaar & van Gelder 1980). The greater variability in the onset of maturation in females is probably a result of their taking at least a year longer to mature than males, so allowing further factors to influence the maturation process.

The histograms of the combined age-distributions illustrate the interaction of two processes, mortality and maturation. These can be represented as two curves that combine to produce the age-distribution found for the toads (Fig. 4). At younger ages, the age-distribution is most affected by the age at which the toads mature, whereas at older ages mortality determines the shape of the age-distribution.

Toads appear to display a classic "*r*" strategy, with high initial mortality as tadpoles and then a fairly steady mortality rate once maturity is reached. From the declining phase of the age-distribution the annual mortality rate is about 50% for males, which agrees well with a mortality rate of 48% calculated for the same population from mark-recapture data (Gittins, 1983). For females, an annual mortality rate calculated from 6 to 9 years is about 70–75%, which again agrees reasonably well with a figure of 60% from the mark-recapture data.

The size of toads in a particular age-class may vary considerably (Gittins, Steeds & Williams, 1982; Hemelaar, 1983), and there does not appear to be a close relationship between length and age for the toads at Llandrindod Lake. Hemelaar (1983) found some indication that the average length increased in successive age-classes but high variability tended to mask this trend.

It is likely that length in toads is most dependent on growth rates during the immature phase, and good feeding conditions are essential if maximum size is to be attained. If this hypothesis proves to be correct it is still possible to have multi-modal age-distributions of adults, but each mode would represent a particular cohort, although the larger sizes may not represent the oldest animals, but ones that have enjoyed good feeding conditions while young.

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