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9. Letters to the Editor are published at the Editor's discretion. Letters should be titled and the name and address of the correspondent given at the end.
10. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.
11. Proofs should be returned to the Editor by return post. Alterations must be kept to the correction of errors; more extensive alterations will be charged to the author.
12. Although minor faunistic lists will not be published, the Editor may deposit such papers with the Librarian of the *British Herpetological Society* and publish a notice to this effect in the Journal.
13. Offprints (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.
14. Papers submitted will be seen by two referees. In general a decision on the acceptability of a paper for publication will be sent to the author within seven weeks of its receipt.
15. In order to encourage the receipt of papers on British species and on the captive breeding of reptiles and amphibians, such papers when accepted will be published in the next available issue of the Journal.

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CONTENTS

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EDITORIAL REPORT FOR 1977

Papers published	1977	1976
Pages published	91	80†
Number of papers	18*	16
Time between receipt and publication (months). Median (range)	23.5 (2–26)*	17.5 (7–26)

* Affected by issue on amphisbaenians.
† Size changed to A5 in December.

Papers received	From U.K.	From abroad	Total
Number received	5	14	19
accepted	3	5	8
transferred to letter	1	0	1
rejected	0	7	7
awaiting decision	1	2	3

Two papers were received on British species

Paper awaiting publication 21

Referees The Editor is most grateful to the following who refereed papers in 1977: E. N. Arnold, T. J. C. Beebee, D. Blatchford, J. E. Cooper, E. Elkan, A. G. C. Grandison, I. F. Spellerburg, M. Whitear, F. B. P. Wooding.

Royal Society Grant A publications grant of £200 has been received from the Royal Society.

ANNOUNCEMENTS

In order to encourage the receipt of papers on British species and on the captive breeding of reptiles and amphibians, such papers when accepted will be published in the next available issue of the journal.

BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 689–693

A QUANTITATIVE STUDY OF METAMORPHOSIS IN THE NATTERJACK TOAD, *BUFO CALAMITA*

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(*Received* 18 *October* 1975)

INTRODUCTION

The British population of the natterjack toad (*Bufo calamita*) is considered to be in danger of extinction, and is protected by law. One of the major causes of its decline, especially in dune habitats, has been the loss of suitable breeding pools (Beebee, 1973, 1975; Prestt, Cooke and Corbett, 1974). Attempts to restore pools of maximum value to the population in areas still occupied by adult natterjacks should benefit from data on the requirements for metamorphosis, and this paper is a preliminary investigation of metamorphosis in this species.

METHODS

A single pool was constructed to the design originally outlined by K. F. Corbett (personal communication). A shallow excavation was made and lined with 500-gauge commercial clear polyethylene sheeting over two layers of newspaper. The pool, approximately of uniform depth (8 cm) and rectangular in shape (4 by 0.9 m), was totally unshaded. It was filled with tapwater which in the particular locality contains >50 parts per million (>1.25 mM) calcium and is "hard". Losses due to evaporation during the developmental period were made good with tapwater. The pool was kept clear of all forms of higher plants, although algae (especially *Spirogyra* spp.) flourished. The filamentous algae were removed daily to restrict growth. Aquatic predators, including water scorpions (*Nepa cinerea*), water boatmen (*Notonecta*), water beetles (*Colymbetes fuscus*), dragonfly larvae (*Odonata*) and newts (*Triturus vulgaris*) were seen to take tadpoles and were removed as they appeared. No other forms of predation (i.e. by non-aquatic species) were observed.

Control development experiments were carried out, again in tapwater, in plastic tanks, 30 by 75 cm, containing water to a depth of 3.5 cm.

Spawn strings from four pairs of natterjacks were placed in the pool. Their removal from the wild was justified by the imminence of desiccation of the breeding pond. This spawn was from a heathland site, and other heath and dune samples were also reared in tanks. All studies were started within 24 hr of oviposition. The four spawn strings placed in the main pool contained an estimated 7000 viable ova, as judged by colour and preliminary elongation of the embryos. An additional 4000 dead ova were present in the spawn strings immediately following deposition, a high initial mortality which occurred prior to the spawn being moved to the experimental pool; the move itself caused no detectable additional mortality. Large numbers of non-viable ova were only

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observed in this particular natterjack population, and were not seen as a regular feature of the species.

The ova were allowed to develop through to metamorphosis in the pool or tanks. Food was mainly endogenous, in the form of filamentous or other algae. A supplement of commercial rabbit pellets was given to compensate for the removal of the filamentous algae (1 pellet/tadpole week in tanks or 50 pellets/week in the pool). Animal protein in the form of meat scraps was offered at intervals to determine at what stage this type of food can first be used (see below). *Daphnia*, which proliferated in the pool, seemed to escape consumption, as far as could be ascertained by direct observation.

All development was subject to prevailing external climatic conditions.

Larvae were considered to have undergone metamorphosis, and were removed, on the appearance of the fourth limb. During the emergence period these animals were caught and counted daily, and later released near the original spawn site following tail resorption.

RESULTS

The progress of development, including the first use of animal protein and the numbers of metamorphoses per day, is shown in Fig. 1. The time taken to complete metamorphosis by individuals spawned within 24 hr of each other varied by a factor of

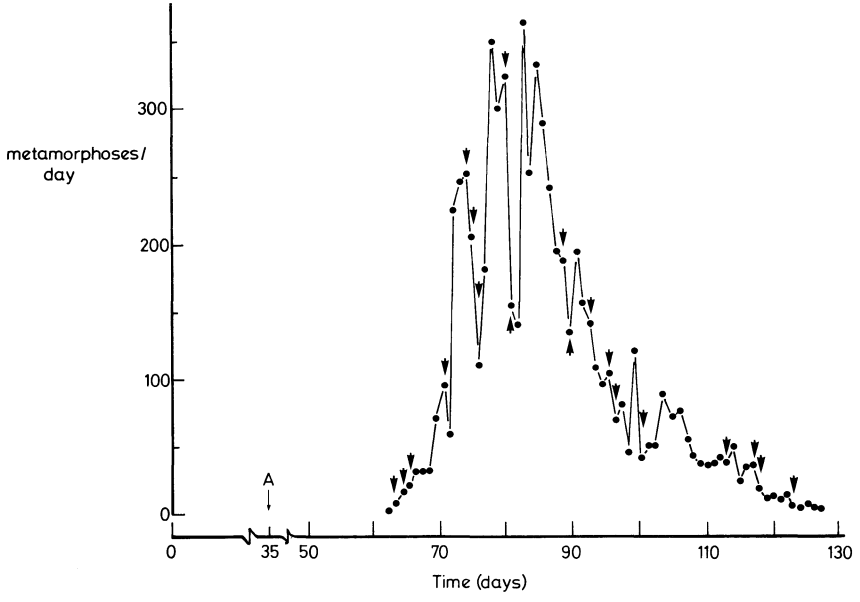

FIG. 1. Time-course of metamorphoses in the experimental pool. The arrows indicate cool, cloudy days with water temperatures not attaining 25°C. "A" shows that day on which animal protein was first eaten. Day 0 is the date the spawn was laid.

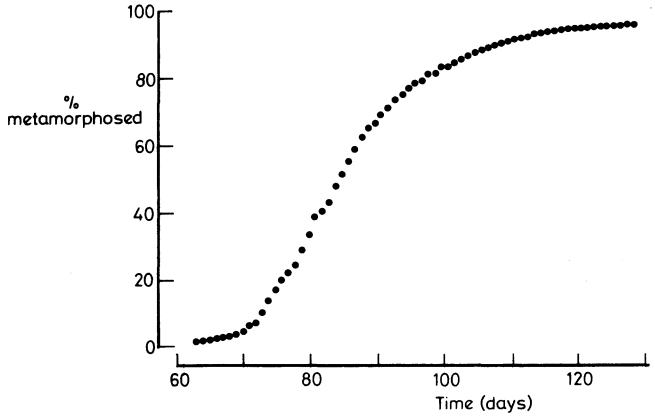

FIG. 2. Cumulative percentage of metamorphoses in the experimental pool (as a percentage of the number of viable ova). 50% had metamorphosed by day 84 (7 July) and 75% by day 94 (17 July). Day 0 is the date the spawn was laid, and day 60 is 13 June.

two, i.e. between 9 and 18 weeks. The spawn, which was deposited very early for *B. calamita* on heathland (16 April) and in a cold spring, hatched over a period of 5–8 days. Animal food was first taken on day 35.

Metamorphoses occurred between days 62 and 127, reaching a peak on day 83 (6 July). During the most active period of metamorphosis, three distinct peaks of emergence were apparent, around days 72–74, 78–80 and 83–85. The troughs between these peaks correlated with periods of relatively low temperatures, and it became apparent that cool, cloudy days markedly inhibited the number of metamorphoses occurring during this main emergence period. This relationship tended to disappear later in the season. No doubt many factors determined the shape of this skewed normal distribution, but temperature seems likely to have been one of them.

By expressing the data as an ogive, the cumulative frequency of completed metamorphoses (as a percentage of the number of initially viable ova) against time (Fig. 2), it can be seen that 50% had emerged by day 84 and 75% by day 94 (17 July). 93% of the viable ova survived to metamorphosis, the last emerging on 19 August; this is a minimum figure since a few may have escaped observation and capture.

The effects of tadpole density and time of oviposition on the rate of development were also investigated (Table I). From these data it is clear that reducing the density of the larvae trebled their weight at metamorphosis, but did not greatly affect the time required for development. Spawn laid by dune-dwelling natterjacks on 4 May, some 18 days later than the heathland sample, metamorphosed at around the same time, i.e. considerably more quickly.

Table II shows the temperatures recorded from various parts of the experimental pond, a deeper pond nearby, and some Lancashire dune slacks on sunny days in May and June. Whereas virtually all water temperatures in the experimental pond were greater than 25°C at noon, only the exposed surface layers and shallows of the deeper pond attained such high temperatures.

Among the developing larvae, six tadpoles with various degrees of pallidity were observed, and these were reared separately. Of these six, four were total albinos and

TABLE I. Effect of population density on metamorphosis

Tank	Source of spawn	$M_{\frac{1}{2}}$	Number of tadpoles/l	Mean weight of young toads (mg)
Pool	Heathland	84	25	46
Tanks	Heathland	73	3	155
Tanks	Dunes	55	3	150

$M_{\frac{1}{2}}$ is the time taken for 50% of the population to metamorphose (days).

Four batches (four tanks) of each population were reared, with 20 tadpoles in each. Survival in the tanks was better than 95% in all cases. Weights are means from samples of 10 newly-metamorphosed toads.

TABLE II. Temperature recorded in pools. See text for details

	1	2	3	4	5	% > 25°
Rearing pool	29	—	—	27*	—	100
Control pool	29	28	22	23	17	10
"Slack"	28	28	23	25	18	50

Temperatures (°C) were measured at noon on bright sunny days, and they were usually consistent ±1°. Five measurements were made during two days. 1. Surface water in shallows (<8 cm deep), 2. Surface water in deeper regions (>15 cm), 3. Surface (shallow) water in shade, 4. Water 10 cm down (*8 cm), 5. 50 cm down and under vegetation. % > 25° is the estimated percentage of the total water volume >25° (average of four slacks).

two of the four survived to complete metamorphosis. The small toads were entirely white except for pale yellow vertebral stripes and red tips to the warts; they survived some weeks in captivity. White larvae have also been observed in dune populations (J. Griffin, personal communication).

Growth rates after metamorphosis were rapid. Some of the young toads released in September (metamorphosed in June at 5–7 mm) had attained 32 mm in length.

DISCUSSION

It is always difficult to reconcile experimental observations with real field phenomena. Certain factors are clearly different, for example, the types and availability of food differences in predation. Even with these reservations in mind, however, it should be possible to draw some meaningful comparisons and emerge with some useful guidelines on the physical variables more directly involved. Other ecological factors may be expected to affect the overall survival rate, but hopefully not the general pattern, and several clear facts do emerge. For a high proportion of metamorphic success, surface water must be maintained until at least the end of July. Of course there will be many environmental factors which will differ in the field from place to place and from year to year but virtually all of these will tend to prolong the period over which water must be present continuously in the slack basins. The year of the experiment (1975) was characterised by a very warm summer, and in cooler years

or more northerly climes the period may need extension. It would appear that the density of tadpoles will probably have little effect on the time required for metamorphosis to be completed. The spawning period was also unusually early; natterjack ova deposited later develop more quickly (presumably because the colder spring weather is avoided) and water requirements in the summer months may not therefore be extended appreciably as a result.

May and June are the months of lowest rainfall in the British Isles; precipitation increases through July and August, and the most critical period certainly lies in June when the first metamorphoses are likely to occur at the time of the most likely period of drought. Also of significance in its possible effects on the period needed for development is the asynchronous spawning pattern typical of natterjack populations in the wild. Spawn deposition usually continues over a period of many weeks (Smith, 1951). Under such conditions, well developed tadpoles exert inhibitory effects on the growth of younger ones; a similar effect occurs if *B. bufo* tadpoles are present (Heusser, 1972). Thus development will be delayed even further, and the presence of surface water until the end of July is probably a minimum requirement.

Although density did not greatly affect development time, it seems possible that the larger toads emerging from low-density conditions may have a better chance of survival in the wild. Until more is known of this factor, it would seem sensible to minimise densities by maximising the area of water available.

Natterjacks are normally regarded as spawning in shallow, temporary pools (Smith, 1951), and this may be due to the high temperature required for development (25°C) (Mathias, 1970). It is clear from the data that a high proportion of the water in shallow pools attains 25°C on summer days. An apparent effect of ambient temperature on metamorphosis was observed.

It would seem that ponds created for the conservation of the natterjack in the wild should take these considerations into account at the design stage. The indications are that unshaded pools with extensive shallow areas shelving deeper to compensate for drought periods (i.e. saucer-shaped), and as large as possible, would be ideal.

ACKNOWLEDGEMENTS

We thank other members of the Conservation Committee of the British Herpetological Society. Especially Mr V. Taylor, Mr K. Corbett and Mr. J. Griffin, for their advice and assistance.

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NEWTS IN THE NEW FOREST

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(*Received* 1 *January* 1976)

SUMMARY

Surveys of newts in the New Forest were made in 1974 and 1975 using a standard technique, and covered some seventy-five waters. Environmental factors affecting the three British species were examined, as they affect distribution. While the netting technique used is probably adequate to discover *Triturus vulgaris* and *T. helveticus*, searching with a torch after dark is a better way of locating *T. cristatus*.

INTRODUCTION AND METHODS

A study of newts in ten ponds in the New Forest was carried out by Creed in 1960 and 1961 (Creed, 1964). In 1974 and 1975, these ponds were re-surveyed and a number of additional ponds were also examined. In all, seventy-two ponds were netted, as well as two lengths of stream and a shallow, flooded ditch. Details of the 1974 results have been given by Cooke and Frazer (1976). Newts were found in thirty-eight ponds, one stream and the ditch. In 1974, the maximum depth of water was determined accurately, as well as pH, and chemical analysis of the water in each pond where newts were present was carried out. In 1975, only the water depth was estimated visually. In both years, pond size, vegetation, bottom quality and the presence or absence of invertebrates were noted.

In 1974 and 1975, netting took place for a standard period of 15 man-minutes per pond. In order to assess the efficiency of this sampling method, repeated samples were taken on one or two occasions in 1975, and mark-recapture studies made. Marking was by clipping a toe on the left hind foot. Methods in 1974 have been described by Cooke and Frazer (1976).

RESULTS

Overall figures on the occurrence of newts are shown in Table I.

The ponds include one covered by Creed in 1960–61, which was dry when examined in 1974. Other changes have also occurred in some of the ponds through the

TABLE I. Numbers of waters examined, and the occurrence of newts, 1960–61 and 1974–75

	Ponds	Streams	Ditches
Number examined	73	2	1
Without newts	32	1	0
With newts	41	1	1
With *T. cristatus*	8	0	0
T. vulgaris	20	0	1
T. helveticus	34	1	1

TABLE II. Changes in water pH in three ponds between 1960–61 and 1974

Pond	1960–61	1974
Pilley	>8	6.2
Balmer lawn	7–8	5.8
Burley—clayfields	8	6.9

years. Understandably, depth alters with time, season and rainfall, and changes in the pH of the water become noticeable. Creed (1964) found intra-seasonal changes in pH in some of her ponds, while marked differences appeared in some ponds between 1960–61 and 1974–75; particular examples are shown in Table II.

It was noticed that the great-crested or warty newt (*T. cristatus*) occurred particularly in ponds with banks of water-buttercup (*Ranunculus fluitans*) though in one case a female was found in a pond with hardly any weed at all; fairly deep water was usually favoured by this species. The two smaller species were found more widely than *T. cristatus* (Fig. 1) but this different distribution has been shown to depend on the metallic ion content (and hence pH) of the water, the palmate newt (*T. helveticus*) being capable of living under more acid conditions than the smooth or common newt (*T. vulgaris*) (Cooke and Frazer, 1976). At pH values below 3.9, no newts were present, and invertebrates were usually scarce.

With repeated samplings of the one ditch examined, comparable numbers of newts were obtained during three successive periods, covering the whole area twice. The two smaller species were present, and mark-recapture studies in 1975 for a 46 m stretch, roughly 1.2 m wide and up to 30 cm deep, gave approximate populations of 57 males and 100 females of *T. vulgaris*, and 81 males and 57 females of *T. helveticus*, i.e. a total of 295 news or one newt per 0.2 m^2 of water surface.

T. cristatus was not readily taken by netting, even in ponds where it was known to occur. Examination by torchlight of these ponds after dark showed that while one or two newts might be netted in a single sample, between 25 and 50 might be seen near the edge in a single circuit of the pond. Only a few individuals of the other two species could be seen in this way, and they were very wary of the light. The light was generally

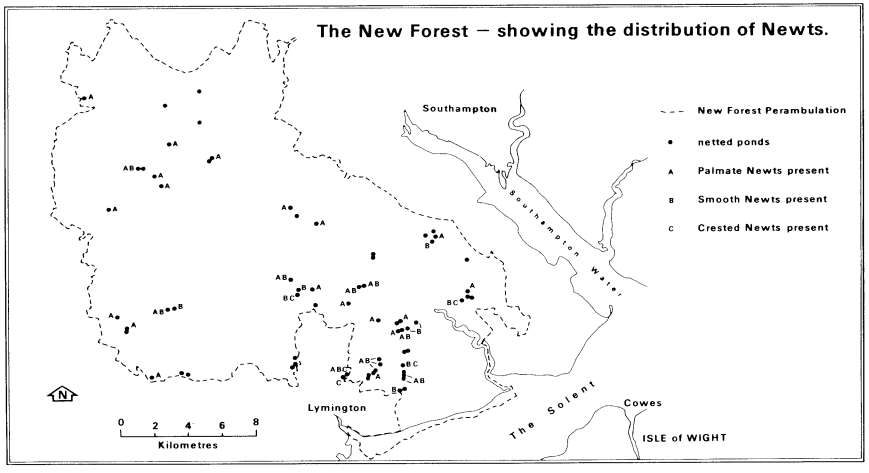

FIG. 1

TABLE III. Numbers of newts netted 1974–5, relative to waters where they were found

Newt species	Newts taken: Males	Females	Total	Number of waters with newts
T. cristatus	4	110	114	5
T. vulgaris	47	40	87	19
T. helveticus	96	78	174	25
Totals	147	228	375	49

ignored by *T. cristatus*, and these newts, of both sexes, would stay motionless for 10 min or more; some females were found laying on banks of waterweed at this time.

In the 1975 survey, overwintered larvae of one of the smaller species were found in five ponds, one of which normally only holds water temporarily after rain. This pond was probably dry in May 1974, and one other (an artificial pond) is known to have been cleared out at that time.

Relative numbers of adult newts taken by netting in 1975 are shown in Table III. Statistically, the proportions between the sexes did not differ between the two smaller species (*T. vulgaris* and *T. helveticus*) but where they were present, rather more palmate newts appear to have been caught in a standard period of netting; exactly twice as many palmates as smooth newts were taken. This may not mean that the palmates are more plentiful, since in one ditch some 30 smooth newts and 30 palmates were collected over 45 min on 29 April 1975, and a 38-min sample the next day yielded 4 and 12 respectively, i.e. only one-third of the numbers expected.

Tadpoles of *Rana temporaria* and *Bufo bufo* were found in a number of ponds. Toad tadpoles were found in eleven ponds with water pH varying from 4.1 to 8.2, but frog tadpoles only in two (pH 6.8 and 7.2) in 1974. In 1975, they were found in another two and three ponds respectively, i.e. totals of 13 and 5 out of the 75 waters investigated.

DISCUSSION

As a method of sampling, it is clear that a standardised netting procedure can give useful results for the two smaller species, but in clear, moderately deep water the presence of *T. cristatus* can be more readily detected by using a torch after dark. Yet the fact that any species is not detected in a brief netting session cannot be taken to mean that it is not present. Fishing the whole ditch on 30 April 1975 only produced 16 newts from an estimated population of 295. Furthermore, one may be fishing in the wrong part of the pond. In pond 54, only ten yards or so from this ditch, no newts could be taken amongst the weed in the deeper water, which looked most suitable. But the two smaller species were both caught in the shallows, presumably where the large fish seen in deeper water could not take them.

The distribution of the ponds and the species found are shown in Fig. 1. While it might be argued that *T. cristatus* was only found in the southern and eastern parts of the Forest, it is necessary to consider this in relation to the small number of ponds where they were detected, and to the gravels in the northern and western parts, where many ponds proved to be too acid for any newts, so that there is a tendency for all the species to be found to a greater extent in the south-eastern part. It has already been shown that *T. cristatus* prefers deeper ponds with no emergent vegetation (Cooke and Frazer, 1976), and these are not plentiful in the Forest.

While the apparent difference in sex ratio in *T. cristatus* and the other species is not statistically significant, it was noticed that females could often be taken if masses of waterweed near the banks were thoroughly netted. The results of repeated netting of the ditch show that repetition of this disturbance could be counter-productive in subsequent catches.

Another problem lies in the disappearance of newts from a pond where they had previously been found. In pond 47, the 1960–61 survey showed all three species, but in 1974 only *T. vulgaris* was found, while in 1975 not even these could be detected. One possibility is that the installation of a nearby camping ground may have affected the newts, but in this rather muddy pond there could merely have been failure to detect newts by the later sampling. On the other hand, an apparently completely suitable pond with clear water at pH 6.9 (No. 46), where the earlier survey showed both *T. vulgaris* and *T. helveticus*, proved to be completely free of them in 1974, although toad tadpoles were plentiful.

Toad tadpoles were markedly more plentiful than frog ones, though this may not necessarily reflect the proportions of adults of the two species in the area. It is known that frogs spawn in shallower water than toads (Frazer, 1953a, b, 1955, 1956) and that even when they spawn in the same pond, different parts of it may be used by the two species (Frazer, 1966). Frogs come out of hibernation at a lower temperature than toads, so that they usually spawn a few days earlier. Furthermore, frog tadpoles are favoured articles of diet for a variety of vertebrates and invertebrates, while there is some evidence (Wassersug, 1971) that toad tadpoles are protected from vertebrate predation by their skin secretions. There is, therefore, a greater chance of predation affecting frog tadpoles more severely than toads by the time the surveys were made. In 1975, one pond (No. 47) where both kinds had been present the previous year, and which had been entitled by Creed the "tadpole pond", had fairly abundant toad tadpoles, but no frog tadpoles. Black-headed gulls (*Larus ribibunda*) were now present. Cooke (1974) has estimated that in one season a pair of *T. cristatus* could eat all the tadpoles from a single clump of frog spawn, and a similar degree of predation could occur from many other vertebrates. It should also be noted that many of the ponds examined are in dry heathland, terrain which is not particularly suitable for frogs.

Two of the ponds examined are of known age. One is the deep Cadman's Pool (pH 8.2) roughly two acres in size, which was excavated in gravel adjacent to woodland in 1966. This contained palmate newts and an abundance of toad tadpoles. A much higher population of palmates was present in pond 37 (pH 4.6) which had abundant invertebrates. This was excavated in 1962 and is separated by a belt of dry woodland from the nearest pond, 1.6 km away. The waters of the Linford Brook pass close to it, but the stream is wide and fast-running—totally unsuitable for newts. How the newts have colonised this pond so rapidly across apparently unsuitable terrain is a matter for speculation, although the dry woodland is undoubtedly less unsuitable for them than open woodland would be. Since the pond is only 13 years old, this is a useful pointer to the speed at which the palmate newt can extend its breeding range under suitable conditions.

During the 1974 sampling, there was some difficulty in the identification of the females of *T. vulgaris* and *T. helveticus*. While the majority were easily distinguishable, a small number appeared by their colour to be *T. vulgaris*, yet their throats were spotless except for one or two minute dark dots. However, when handling newts for marking in 1975, it was clear that the female palmate has short toes, like the male of

that species, while the female common has longer toes like the male of her own species. Once the two have been seen, there can be no further confusion between them.

Among the newts seen in 1974 were two female palmates bearing a skin infection. There is no evidence about the causative organism or mode of infection, but the water was neutral in reaction (pH 6.7 and 7.3). These were the only two noted among 275 newts handled in 1974–75.

The behaviour of newts after dark differed according to species. *T. cristatus* was found stationary in large numbers in clear water outside the banks of weed, about as soon as it was dark. Females were to be found, particularly, very near the shore, where they might remain motionless for half an hour or more. Some females lay on the surface of weed, where a few were seen to be depositing eggs. Males were also present in open water, but were more easily scared by light than females. Although some males were facing other newts, no courtship was seen. The water was very still and it is possible that in these undisturbed waters the majority of newts were waiting for invertebrate prey to fall in or move past. Two females had their heads on the muddy bottom and could have been feeding. Both sexes of the common newt were seen to behave similarly, though in markedly lower numbers. Palmate newts were seen only in small numbers after dark, even in ponds where they were numerous; they did not stay in the same place for more than 15 min or so, but again moved only very slowly unless disturbed by the light. In a steep-sided concrete garden pond, females came to lie within a few cm of the surface, over masses of vegetation, or could be found near the surface on the vertical concrete sides, where weed covered the rockwork. Newts of this species were easily scared by light from the torch.

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 701–705

NEUTRAL RED DYE AS A MARKER FOR TADPOLES

A. S. COOKE

Monks Wood Experimental Station, Abbots Ripton, Huntingdon

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INTRODUCTION

Recently, neutral red has been advocated as a satisfactory agent for marking tadpoles in order to obtain population estimates (Herreid and Kinney, 1966; Wijnands, 1972; Guttman and Creasey, 1973). The technique involves catching and counting a sample of tadpoles, staining them red, releasing them, and catching another sample before the red colour fades; from the proportion of red tadpoles, an estimate of the total population is obtained by the Lincoln Index method.

Staining procedures used by various authors are shown in Table I. Only Guttman and Creasey (1973) mentioned any harmful effects of treating tadpoles with the dye.

TABLE I. Staining procedures for tadpoles used by various authors

Authors	Species	Concentration of neutral red (ppm)	Time in dye (min)	Persistence of red colour
Herreid and Kinney (1966)	*Rana sylvatica*	500	30	About 1 week
Wijnands (1972)	*R. temporaria* and *R. arvalis*	250	15	Not stated
Guttman and Creasey (1973)	*R. clamitans*	20 or 40	180	At least 10 days

They found that 9% died, but concluded that staining was, nevertheless, a better technique than fin clipping for estimating populations.

The present work was designed to test the suitability of neutral red for estimating the population of common frog (*Rana temporaria*) tadpoles.

METHODS AND RESULTS

TREATMENT WITH 250 PPM NEUTRAL RED FOR 15 MIN

In May 1974, the procedure of Wijnands (1972) was used to estimate the population of common frog tadpoles in a stock pond. By repeatedly counting the number of tadpoles visible, it was estimated that between 100 and 200 were present. On day 1, 50 tadpoles were caught, dyed red (in 1 l fluid) and returned to the pond. Throughout the trials, tadpoles were caught in a handnet; rapid sweeps were made through regions which tadpoles frequented in good numbers. On day 2, a further sample was caught, of which 17 out of the 50 were red. This gave an estimated total of 150, which agreed well with the visual estimate. However, many of the dyed tadpoles had wavy or damaged tail fins, and several appeared emaciated. Tadpoles were returned to the pond after this

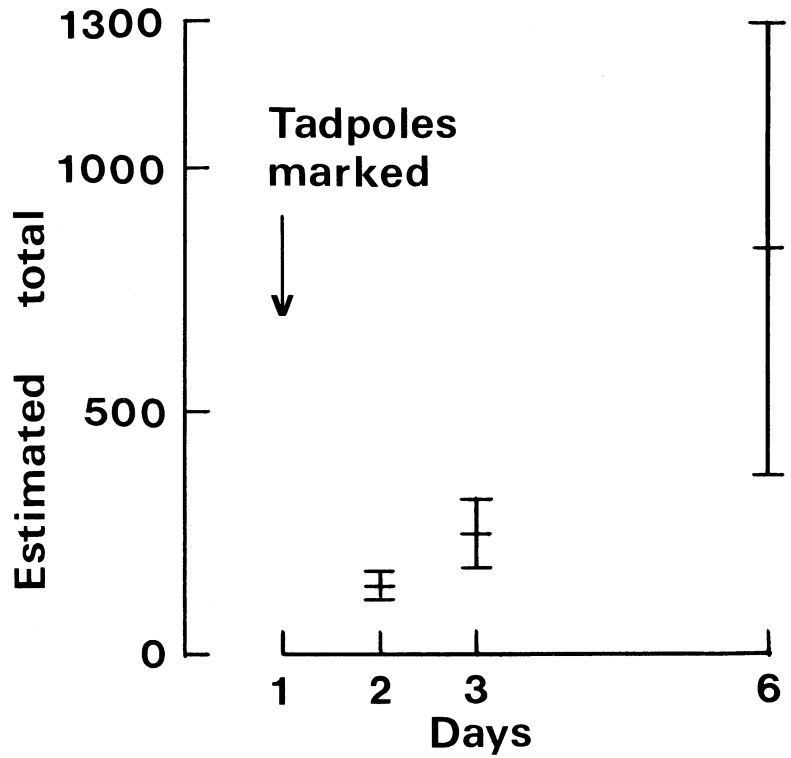

FIG. 1. Estimated total numbers of tadpoles present in a stock pond. Standard deviations are indicated by vertical lines. On day 1, 50 tadpoles were marked by immersion in 1 l 250 ppm neutral red for 15 min. Based on visual estimates, 100–200 tadpoles were believed to be in the pond on day 1.

and subsequent samplings. On day 3, another sample of 50 was netted; this time only 10 were red (estimated total = 250). Finally, on day 6, another sample of 50 tadpoles contained only 3 red ones (estimated total = 800). Despite a further search, no more red tadpoles were found. Thus the number of red tadpoles appeared to decrease steadily and so the estimated total increased. These results are shown in Fig. 1, standard deviations being calculated by the method of Bailey (1952). Possible explanations for the findings include: (i) the colour faded, (ii) the red tadpoles were dying or hiding, (iii) the marked tadpoles were being selectively eaten by smooth newts (*Triturus vulgaris*) in the pond, or perhaps by the normal tadpoles. Attempts were made to test these possibilities in the laboratory after staining in 250 ppm neutral red for 15 min.

When kept in tanks, the red tadpoles did not die, nor did they tend to hide more, nor were they eaten by normal tadpoles; the red colour persisted for at least 5 days. Newt predation was not satisfactorily tested since the newts used showed more interest in trying to get out of the tanks than in catching the tadpoles, and no tadpoles were caught. Although none died, the red tadpoles displayed symptoms of poisoning. Each time they were returned to the water after being netted they showed much tail lashing

and body twisting, similar to the "frantic" behaviour phase of tadpoles treated with DDT (Cooke, 1971), except that movements were not so rapid. This behaviour persisted for only a few minutes at a time. Also, 20% of the treated tadpoles had the tips of their tails missing.

Therefore the fate of the red tadpoles in the stock pond remains unexplained. It is conceivable, however, that since the tadpoles in the pond were probably exposed to greater stresses than those in the laboratory (emaciated tadpoles were seen in the pond but not in the tanks) some of the treated tadpoles may have died from poisoning. The rather more severe treatment of Herreid and Kinney (1966) is apparently lethal to *Rana temporaria* tadpoles (G. Moore, personal communication). An alternative explanation is that the red tadpoles were selectively eaten by the newts. The fact that warty newts (*T. cristatus*) will preferentially prey on hyperactive, DDT-treated tadpoles (Cooke, 1971) lends support to this suggestion. The dyed tadpoles were in the size range of snout-anus length 6–9 mm (weight about 100 mg), and there were at least seven adult smooth newts in the pond. The 50 dyed tadpoles could have been accounted for in about a week (see Cooke, 1974).

TREATMENT WITH 250 PPM NEUTRAL RED FOR 5 MIN

On 28 May 1975, 2266 tadpoles (hind limb buds and paddles stage) were released into the stock pond. These tadpoles had previously been kept at a similar density under predation-free conditions, and mortality from hatching was on average only 1% per week. The tadpoles were rather more advanced than those used in 1974 (and therefore

TABLE II. Details of mark/recapture trials with neutral red. 200 *Rana temporaria* tadpoles were dyed in each trial. Total number of tadpoles in the pond was assumed to be 2266. All tadpoles were returned to the pond after being counted

Time after treatment (hours)	Total number caught	Number of red tadpoles in catch	Estimated total ± S.D.	Estimated / actual
250 ppm neutral red for 5 min				
0.5	170	14	2400 ± 600	1.06
5	211	29*	1500 ± 300	0.66
22	259	40***	1300 ± 200	0.57
48 (a)	225	28	1600 ± 300	0.71
(b)	185	12	3100 ± 900 (2900 ± 700)†	1.37 (1.28)†
(c)	239	21	2300 ± 500 (2200 ± 400)†	1.01 (0.97)†
48	Total 649	Total 61	Mean 2100 ± 300	Mean 0.93
250 ppm neutral red for 1 min (started 2 days after above trial finished).				
0.5	218	16	2700 ± 700	1.19
2	243	20	2400 ± 500	1.06
21	220	17	2600 ± 600	1.15

* Significantly higher number than expected, $\chi^2 = 6.37$, $P < 0.05$.
*** Significantly higher number than expected, $\chi^2 = 14.25$, $P < 0.001$.
† Numbers in brackets take previous catch(es) at 48 hours into account. Preceding numbers do not.

less liable to be eaten by the smooth newts), and losses due to mortality during the six days of these trials can probably be safely ignored.

A sample of 200 tadpoles was dyed red in 1 l of 250 ppm neutral red for 5 min on 29 May, and samples of about 200 were caught at certain times (Table II). Knowing how many tadpoles were in the pond, the accuracy of each estimated total could be tested. After 5 and 22 hr, significantly more red tadpoles were caught than would have been expected, leading to very low estimates. After 48 hr, three successive samples of about 200 were taken before the tadpoles were counted and returned to the pond. The first recapture again gave a very low estimate, but the second and third did not. Soon after the tadpoles had been dyed, some were observed to be more mobile than normal, and these tended to come to the surface more frequently, apparently to breathe. Thus, although the catch after half an hour gave a good estimate, the other results strongly suggested that some of the red tadpoles were much easier to catch than the untreated tadpoles. No aggregates of red tadpoles were observed. The red colour became very faint in three days.

Treatment with 250 ppm Neutral Red for 1 min

On 2 June, a further sample of 200 tadpoles was dyed in 1 l of 250 ppm neutral red for 1 min. Subsequent recaptures (Table I) gave estimates of the total number that were rather high, but not significantly so. By 21 hr, the red colour was barely discernible, being restricted to a faint suffusion on the belly and on the ventral fin.

DISCUSSION

Whatever the reason for the loss of red tadpoles after treatment for 15 min, the observations suggest that the previously recommended staining treatments are too severe for *Rana temporaria* tadpoles, particularly if population estimates are made on several occasions during one season. Treatment with 250 ppm neutral red for 15 min may well give a reasonable population estimate based on the catch the day after staining, but if all the red tadpoles die within a few days, the staining operation may be a significant mortality factor. If one fifth of the tadpoles are netted, stained and subsequently die during each estimation, then four such estimates made during a season could kill up to 60% of the original population.

Reducing the treatment time to 5 min apparently leads to problems because of behavioural changes. Treatment with 250 ppm neutral red for 1 min seems to be more satisfactory, but the recapture should be made within 24 hr. Should a mark be needed that persists throughout the larval stage, Seale and Boraas (1974) have recently developed a method involving injection of a non-toxic, oil-soluble dye into the tail fin. This has been employed successfully on several American species, but for simple mark-recapture estimates, the technique suffers from being time-consuming and liable to injure the tadpoles.

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I am grateful to Dr E. Pollard and Dr R. K. Murton for commenting on the manuscript.

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 707–709

AVOIDANCE OF SALINE SUBSTRATES BY JUVENILE NATTERJACK TOADS, *BUFO CALAMITA*

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In recent years an important cause of breeding failure in populations of the natterjack toad, *Bufo calamita* Laurenti, in coastal areas of northwest England, has been the drying up of the temporary pools in which spawn is laid, as observed by Smith, Harris and Hancock (1974) and Beebee (1976). During an investigation into the breeding status of a small saltmarsh colony in 1977 approximately 300 larvae were rescued from a shallow pool just before it dried up. Since there was no suitable water body at the site to which the larvae could have been transferred, they were grown through to metamorphosis in the laboratory in a large tank of running tap water. Approximately 250 toadlets were subsequently returned to the site. By the time that most individuals had completed metamorphosis, at the end of June, only one of the pools that were not regularly inundated with saline water had not dried out completely, and in that the salt concentration had risen to 21% of that in sea water (in this paper salinities are expressed as percentages of sea water containing 35 parts per thousand by weight of dissolved salts). This salinity is well below the upper lethal limit for adult natterjacks recorded by Mathias (1971), but considerably greater than the upper tolerance level for development of the larvae noted by that author. Accordingly, before returning large numbers of toadlets to the margin of the above pool, the animals' response to saline substrates was assessed.

Salinity choice chambers were made by dividing in half each of a series of 10 cm diameter glass crystallising dishes with an impermeable barrier across the middle, and filling up to the top of the barrier on one side with sand soaked in tap water, and on the other with sand soaked in sea water diluted with tap water to salinities of 23%, 46% and 69% sea water. Salinities were determined by titration against silver nitrate. Both tap and sea water were equilibrated to the temperature of the laboratory, 23°C, before use. Illumination was provided by fluorescent lights positioned directly above the choice chambers. Three newly metamorphosed toadlets were placed on each side in each chamber and their distribution recorded at intervals over an hour, twelve trials being conducted at each salinity. The results (Fig. 1) were analysed by means of the χ^2 test described by Bailey (1959) to determine whether or not the distribution of the animals differed from that expected if they distributed themselves at random between the two sides.

There was no significant aggregation at the end of an hour on either side in the 23% choice chambers ($\chi^2 = 0.9$; $P > 0.05$) and for six replicates the mean number of animals on the saline side after a further two hours was the same as that on the non-saline side. In contrast, there was very marked aggregation on the non-saline side after only ten minutes for both the 46% and 69% trials ($\chi^2 = 10.9$, 20.6 respectively; $P < 0.001$).

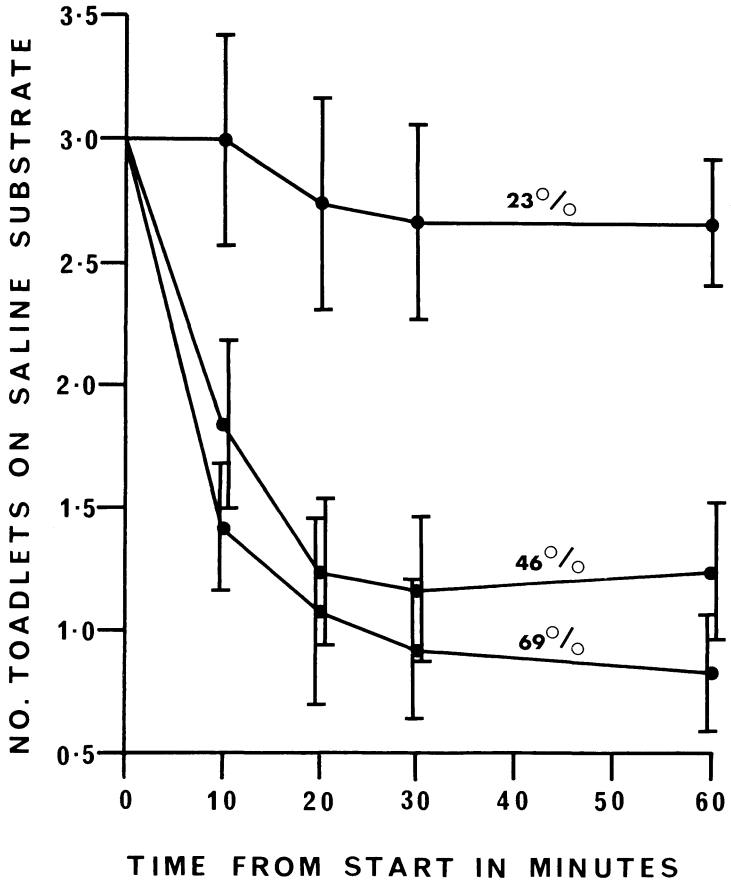

FIG. 1. Movements of *Bufo calamita* toadlets offered a choice between sandy substrates soaked in tap water and saline water. Means of 12 replicates ±S.E. Salinity expressed as % seawater.

At the last sighting of an adult natterjack on the saltmarsh in 1977, the salinity of the water in which it was partially submerged was 22% sea water. Mathias (1971) reported an upper lethal limit for adults, immersed for four days, of 46–50% sea water (equivalent to 55–60% for the Ainsdale sea water that he used, which only contained 29 parts per thousand of dissolved salts). Both observations are based on small samples, but are consistent with the behaviour of juveniles in the choice experiment described here. In view of the results of this experiment, the majority of the toadlets were released at the margin of the only remaining moderately saline pool on the site. It seems likely that on the saltmarsh the toads will tend to congregate in regions of moderate to low salinity and to avoid substrates of salinities much greater than 23% sea water. The relatively high threshold value of the salinity response will clearly be of advantage to toadlets emerging from the water at a time of year when the margins of normally quite fresh saltmarsh pools may have a high concentration of salts.

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 711–717

MORPHOLOGY OF THE HEMIPENIS AND CLOACAL GLAND, AND SEASONAL CHANGES IN THE TESTIS OF THE SNAKE, *AHAETULLA NASUTUS*

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SUMMARY

In *Ahaetulla nasutus* the testes enlarge considerably during the breeding season. The spermatogenetic cycle is almost complete shortly after the males have left their winter quarters. The entire genital system becomes enlarged in March and April during the breeding season. The cloacal glands show no seasonal variation. The anatomy of the hemipenis is described.

INTRODUCTION

Little attention has been paid to the breeding biology of Indian snakes. Therefore in the present studies, the reproductive cycle in male snakes of the species *Ahaetulla nasutus* (Lacépéde) has been investigated.

MATERIALS AND METHODS

Specimens of *Ahaetulla nasutus* were obtained from dealers in Calcutta. Most were kept alive in the laboratory. They were anaesthetised with chloroform, and the lengths and weights of the snakes and various organs recorded. Specimens were fixed in alcoholic Bouin's fluid, Zenker's fluid or TCA. Serial sections of the hemipenis were cut at 12 μm; other sections were cut at 6–8 μm and stained either with haematoxylin and eosin or PAS.

RESULTS AND DISCUSSION

TESTIS

The paired testes are very elongated with the right anterior to, and a little larger than, the left, corresponding to the difference in the relative position of the kidneys (Fig. 1a). No prominent epididymis could be traced. The vas deferens from each side runs posteriorly along the mid-ventral aspect of the kidney and unites with the corresponding ureter to open into the common urinogenital aperture, which in turn opens into the cloacal chamber (Fig. 1b).

The sexual cycle may be divided into four phases—pre-breeding, breeding, post-breeding and non-breeding. During these phases, the results shown in Table I were obtained. It is clear that in the breeding season the testes enlarge considerably to about 5–6 times the weight, 3–4 times the breadth and 2–3 times the length in the non-

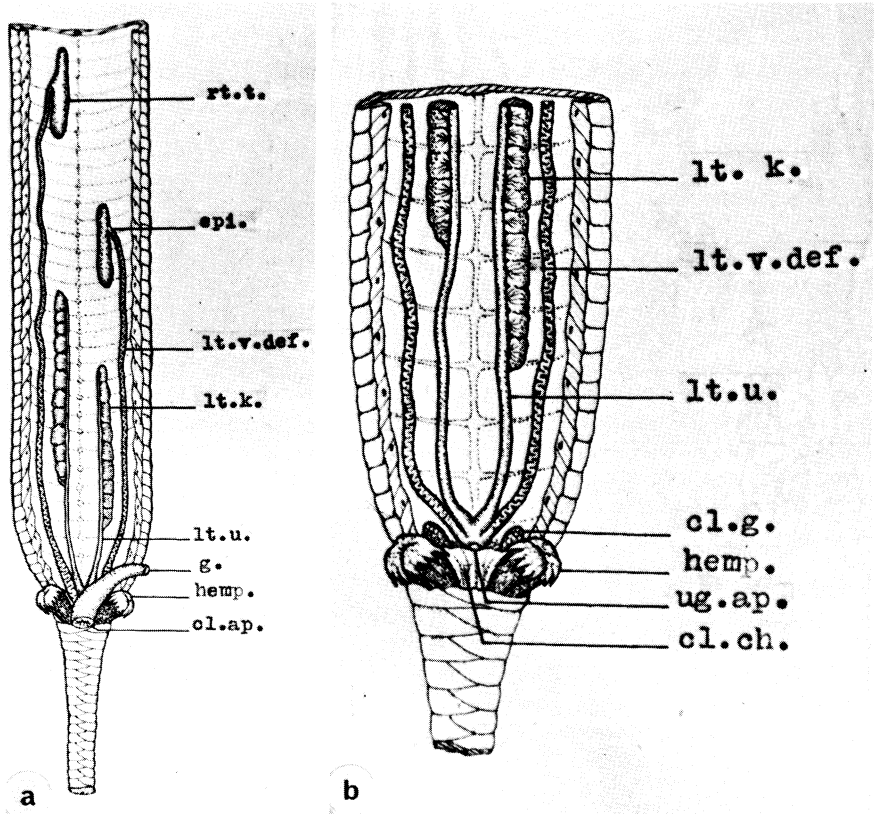

FIG. 1. (a) Male urinogenital organs in *Ahaetulla nasutus*. **cl.ap.**, cloacal aperture; **epi**, epididymis; **g**, gut; **hemp**, hemipenis; **lt.k**, left kidney; **lt.u**, left ureter; **lt.v.def**, left vas deferens; **rt.t**, right testis. (b) Gut is removed and the cloacal chamber is opened longitudinally to show the common urinogenital aperture, **cl.ch**, cloacal chamber; **cl.g**, cloacal gland; **hemp**, hemipenis; **lt.k**, left kidney; **lt.u**, left ureter; **lt.v.def**. left vas deferens; **ug.ap**, urinogenital aperture.

breeding season. During the breeding season, the testes are cylindrical and yellowish-white in colour but during the non-breeding season, they are thread-like and white. Interstitial cells are more numerous in the non-breeding season.

CYCLICAL CHANGES IN THE SEMINIFEROUS TUBULE

Since the structure of the epithelium is at its most simple just after the mating season, the description starts from then (i.e. in May).

May. The tubules have a wide lumen and consist of a single layer of cells which are of two types (Sertoli cells and spermatogonia); in some tubules, ripe spermatozoa are found.

TABLE I. Body and testis size in male specimens of *A. nasutus*. Mean

Phase	Month	N*	Specimen			Testis						
						Right			Left			
			Length	Breadth	Weight	Length	Breadth	Weight	Length	Breadth	Weight
			cm	cm	g	cm	cm	mg	cm	cm	mg
Non-breeding	August–September	10	112	1.2	35	2.0	0.1	10	1.9	0.1	10
Pre-breeding	October–January	15	110	1.2	30	2.3	0.3	50	2.1	0.3	45
Breeding	February–April	12	107	1.2	32	4.1	0.4	60	3.8	0.4	50
Post-breeding	May–July	15	110	1.2	31	1.9	0.1	20	1.7	0.1	15

* Number of animals

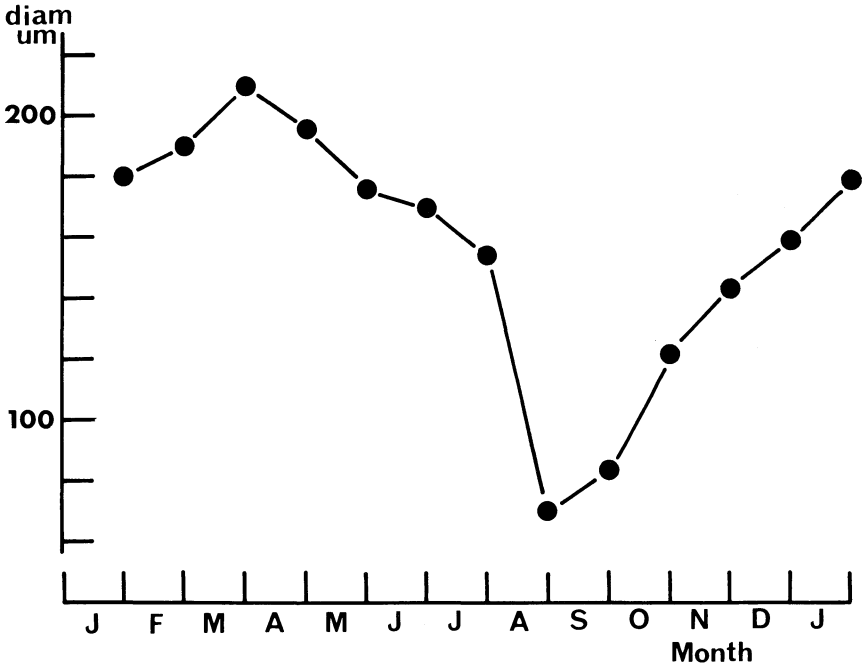

FIG. 2. Seasonal changes in the diameter of the seminiferous tubules. Mean.

June–July. The tubules have contracted, and the diameter of the tubule is somewhat decreased (Fig. 2); there are large intertubular spaces, and spermatogonia form the major cell type.

August–September. The diameter of the tubule has increased (Fig. 2) and the intertubular spaces have diminished. The tubules contain three to four layers of cells and primary spermatocytes in different stages are the most numerous cell type (Fig. 3a).

October–December. The primary spermatocyte is still the most numerous cell type and secondary spermatocytes are only present in small numbers, although there is considerable variation between specimens. In some tubules spermatids are present in very small numbers (Fig. 3b).

January–February. The diameter of the tubule has almost reached its maximum (Fig. 2) and the intertubular spaces have been reduced considerably. The lumen in the tubules has become reduced, and in many specimens it may be obliterated (Fig. 3c). Primary spermatocytes are present but in reduced numbers, and a large number of secondary spermatocytes are present. Spermatids are now the major cell type and, in some tubules, spermatozoa are present.

March–April. Ripe spermatozoa are present and spermatids are by far the most numerous cell type. The discharge of ripe spermatozoa is evident from the increase in the diameter of the lumen (Fig. 3d).

It is clear that the spermatogenetic cycle is nearly complete shortly after the males have left their winter quarters although small numbers of spermatozoa may be found in the ductus deferens throughout the active season.

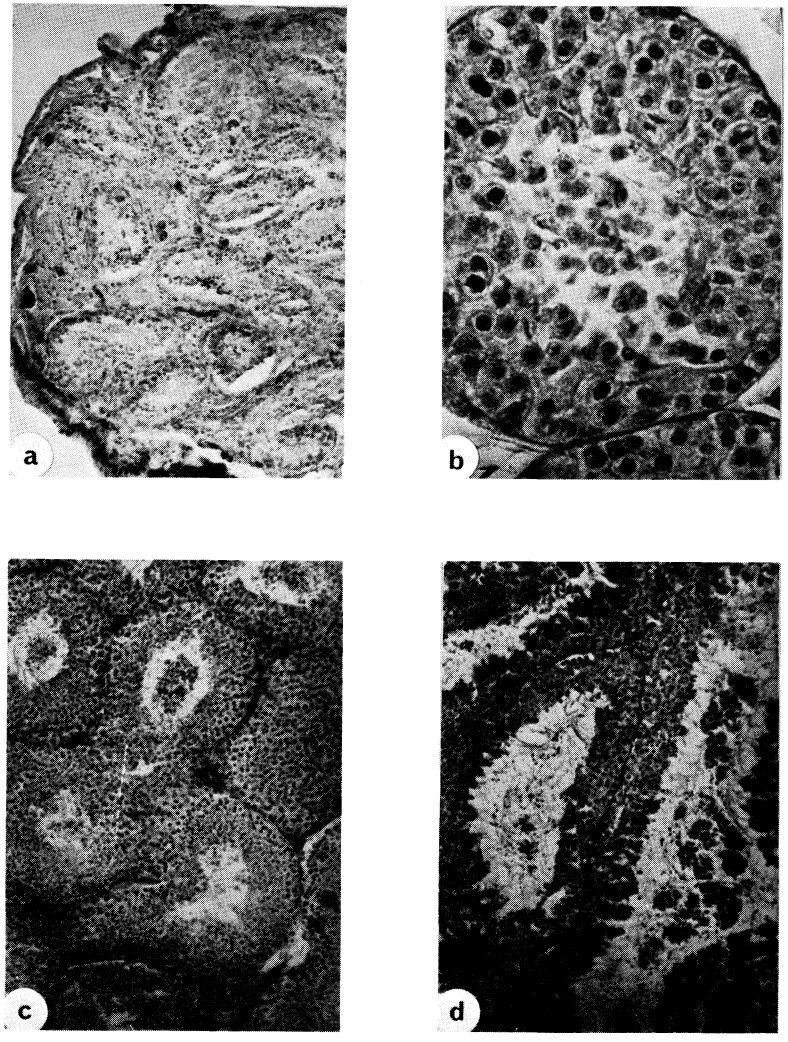

FIG. 3. (a) Testis of *Ahaetulla nasutus* collected in late July. The diameter of the tubule is greatly reduced and spermatogonia are the major cell type. (b) November specimen showing the primary spermatocytes in the tubule, which form the major cell type. (c) Testis in late February. The diameter of the tubule has nearly reached its maximum size and spermatids are now in a majority among the cells. (d) April. Ripe spermatozoa are present in the centre of the tubules.

HEMIPENIS

Each hemipenis opens to the exterior through the lateral margin of the posterior tip of the cloacal opening. Each has a longitudinal groove, the *sulcus spermaticus*, running

through the organ, which serves to transport the spermatozoa from the male into the female. The spermatic groove is bounded by two thick lips. In its fully everted condition the hemipenis is blunt with a spherical head and measures about 1.5 cm in

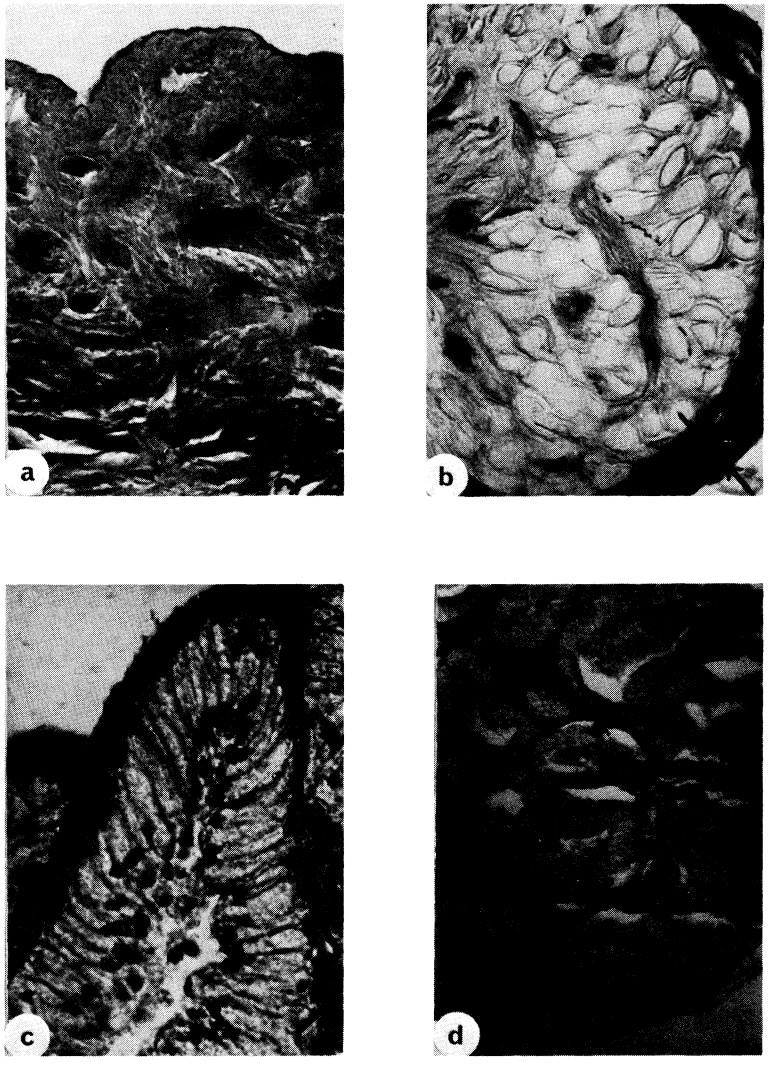

FIG. 4. (a) Structure of hemipenis (T.S.) showing outer sulcate layer with lymph sinuses and inner asulcate layer with blood sinuses. (b) Intense localisation of PAS in the basement membrane surrounding the central retractor muscle of the hemipenis. (c) Structure of a tubule of the cloacal gland. Each tubule is lined by tall columnar epithelial cells with apical nuclei. (d) Intense localisation of PAS in the basement membrane surrounding the tubule and in the intertubular channels of the cloacal gland.

length and 0.5 cm in diameter. At the side opposite to the sulcus and at the tip, two prominent circlets of spines are present. The distal circlet is composed of four to five spines; the proximal circlet has medium-sized spines. The rest of the organ has very small spines and these are often distributed in bunches. The length of each large spine is about 0.4 cm.

The hemipenis is composed of two erectile tissues, the outer sulcate and inner asulcate muscle layers (Fig. 4a). The entire sulcate layer is permeated by lymph sinuses and large blood sinuses are present between the two layers. A large retractor muscle (*musculus retractor penis magnus*) lies in the central space, and the whole organ is covered by a stratified epithelium. Intense PAS-positive material is found within the well-developed basement membrane surrounding the central muscle (Fig. 4b); the sulcate and asulcate layers are also moderately reactive.

The basal segment of the hemipenis is normally everted prior to insertion in the female. Only one hemipenis is used in copulation although both may be partially everted. During eversion, the lymph and blood sinuses become engorged, and after insertion the rise in pressure evaginates the remainder of the organ. The sulcate layer now lies on the exterior surface with the asulcate layer internal to it. Therefore the sulcus spermaticus is on the external surface and is now in a position to carry spermatozoa. The spines become fully extended and anchor the penis in the cloaca. Unless the male retracts the penis, the organ cannot be removed from the female without causing damage to both individuals. Copulation may continue for many hours, even for more than a day.

CLOACAL GLAND

The cloacal glands are oval, flattened bodies situated on either side of the cloaca, into which they empty through small pores. Each gland is about 0.6 cm in length and 0.2 cm in breadth, and the secretion is milky-white. No seasonal variation in structure has been observed.

The gland is alveolar in structure and composed of tubules. The tubules are enclosed in connective tissue, and are lined by a tall columnar epithelium with apical nuclei (Fig. 4c). The average diameter of a tubule is 110 μm (range 80–170). Intense PAS-positive material is apparent in the basement membrane surrounding the tubule and in the intertubular channels; the granular cytoplasm and the cell membrane are also moderately reactive (Fig. 4d). As judged by the PAS stain, an extensive distribution of glycogen is present in the muscles of the hemipenis.

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SEASONAL CHANGES IN THE HISTOLOGICAL STRUCTURE OF THE ULTIMOBRANCHIAL BODY OF THE TOAD *BUFO VIRIDIS*

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SUMMARY

The size and microscopic structure of the ultimobranchial body (paired) of *Bufo viridis* undergoes spontaneous changes during the year. The volume is minimal in mid-summer and its histology reflects a state of rest. There is a gradual increase in size and resumption of follicular formation from September onwards, and maximum levels are reached in December when coagulum formation reaches its peak, indicating renewed secretion of calcitonin. Thereafter glandular size and signs of activity gradually recede.

After hypophysectomy or prolonged administration of calcitonin, the histological structure of the UB closely resembles that of intact glands in mid-summer. After thyroidectomy or prolactin administration the UB is similar to the intact gland in winter. It is tentatively concluded that (a) the pituitary exerts regulatory influences on the UB in which prolactin plays an essential part, (b) the functional state of the UB is related to the seasonal cycle of pituitary activity and (c) to the fluctuations of serum calcium.

INTRODUCTION

The structure and function of the amphibian ultimobranchial body (UB) and its relationship with calcium balance and storage was reviewed by Robertson (1971), who described the UB of *Rana pipiens* to be smallest in June–July and largest in January–February. *Bufo viridis* is similar in this respect, though more drastic histological changes occur. In the present paper the ultimobranchial body of *Bufo* is described by light microscopy using conventional staining methods.

MATERIALS AND METHODS

About ten sexually mature toads (snout-vent length 50–95 mm and weighing 13–80 g) were captured in the vicinity of Jerusalem at the beginning of each month during the year, so as to include the full range of seasonal variations in temperature and humidity: a total of 85 males and 65 females. One pair of specimens was in amplexus, on 1 March 1974 (male 72 mm, 22 g; female 80 mm, 39 g). To eliminate any effects of captivity (Boschwitz, 1967), toads were anaesthetised immediately, or on the day following capture. The region of the larynx was excised and fixed in 10% formalin, Zenkerformol or Bouin-Hollande. Serial paraffin sections 6–10 μm thick, were stained with Ehrlich's haemotoxylin and eosin, with Alcian blue or toluidine blue (Romeis, 1968).

The histology of the ultimobranchial body was compared with that seen in previous experiments involving hypophysectomy, thyroidectomy and treatment with prolactin, calcitonin or calcium (see Boschwitz, 1960a, b, 1969, 1973; Boschwitz and Bern, 1971).

RESULTS

Comparison by the naked eye, of the ultimobranchial glands obtained throughout the year, usually revealed differences in size between the left and right; either gland may be the larger.

The smallest volume was found in June (see also Robertson, 1971). Assuming minimum size reflects minimum activity or even rest, the description begins with the gland of a toad killed in June (Fig. 1).

Histological examination reveals clusters of rather uniform cells, whose nuclei are often situated at the periphery of the cell mass. This arrangement suggests that either a formerly active follicle has been obliterated or a resting one has started renewed follicular differentiation. Occasionally a true follicle with coagulum was seen. There were no signs of secretory activity.

The whole gland is surrounded by a capsule of connective tissue, which includes melanophores and capillaries. Strands of connective tissue separate cell clusters.

Towards September the glands increase in size by hypertrophy and hyperplasia of parafollicular cells, and the development of follicles. However, mitoses were rarely observed; presumably the time of fixation did not coincide with cellular mitotic activity (Bullough, 1952, 1962). Internal connective tissue, capillaries and the surrounding capsule were rather compressed.

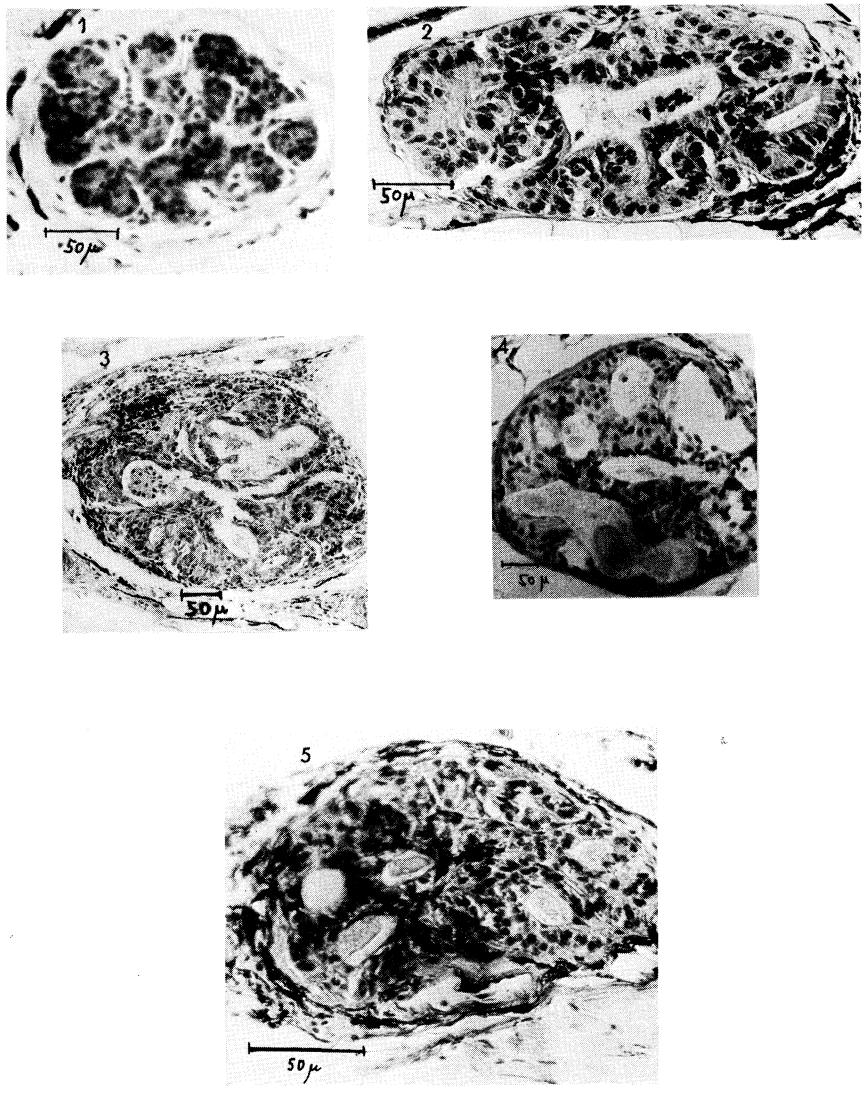
Coagulum formation begins later, reaching its peak in December–January, when the central lumen containing coagulum is larger than previously and is often confluent with the lumina of more peripheral follicles (Figs 2–4). This condition is more pronounced in the larger of the two glands: in other words development, differentiation and secretion proceed at different rates on either side.

The borders of some follicles abut the capillaries of the capsule, permitting discharge and transport of their coagulum, which seems to be more dilute, judged by the much lighter shade of the stain.

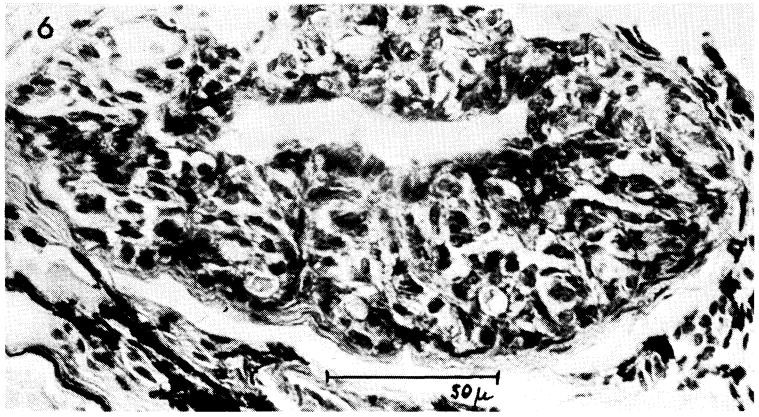
At the end of February and during March the central lumen decreases in size or disappears, but many small lumina persist. The same was found in the two toads in amplexus (Figs 5, 6). In the male the central lumen is narrow, empty and almost obliterated (Fig. 5); in the female no central lumen exists but several small follicles with eosinophilic coagula are present (Fig. 6). In all these animals the bulk of the tissue consists of parafollicular cells dispersed at random. The capsule of the gland is thicker than before and tightly surrounds the parenchyma.

The UB slowly shrinks until June–July (Figs 7–9), when the glands become rather compact, with or without a central lumen; small round follicles containing coagulum of different shades when stained, may persist for some time. Their epithelium is mostly cuboidal; some lumina are bordered only by a crescent of epithelium indicating further regression. The cells of the shrunken follicles become indistinguishable from parafollicular cells which have increased in number.

The capsule has thickened and there is an increase in the amount of connective tissue extending between clusters of parenchymatous cells. Nevertheless, remarkable individual variations in structure of the UB occur throughout the year, even from toads captured at the same time and place.



Figs. 1, 5, 7 and 8: median sections of the ultimobranchial body, which lacks a lumen.
Figs. 2, 3, 4, 6 and 9: median sections of the central lumen. These sections may be paramedian.



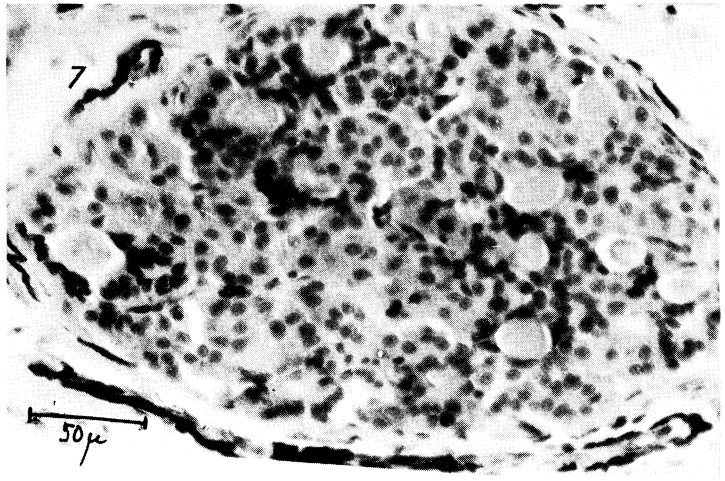


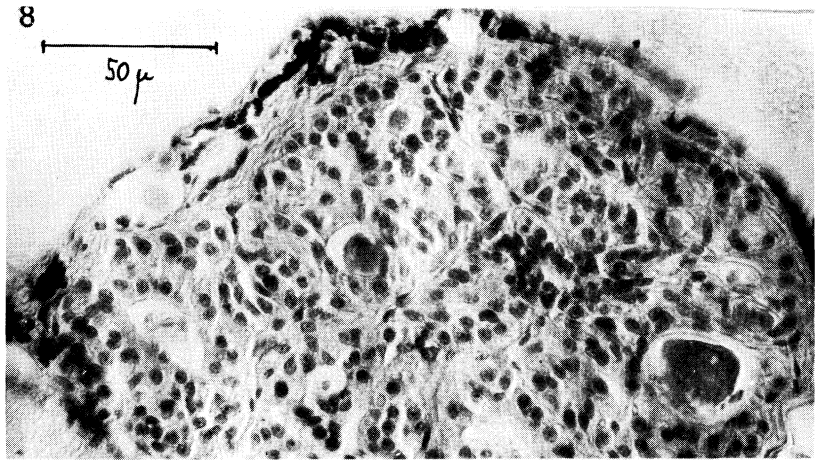
FIG. 1. Toad 72 mm long. Captured and fixed 29 April 1969. Gland almost afollicular. Clusters of cells separated by connective tissue branching from the capsule. At some places nuclei are situated at the periphery of the cell masses resembling occluded follicles.

FIG. 2. Toad 53 mm long. Captured and fixed 25 December 1968. Volume of gland has increased. A central lumen and also peripheral follicles are formed. First signs of secretory activity.

FIG. 3. Contralateral gland of toad of Fig. 2. The central lumen is widened and confluent with several peripheral lumina. Coagulum secretion more abundant than on the other side.

FIG. 4. Toad 93 mm long. Captured and fixed 28 January 1969. Several large lumina and some smaller ones; most of them confluent. Coagulum formation has increased. Between the follicles are parafollicular cells, mainly "stem cells" transitory between their earlier formation and active secretory cells. Gland highly active.

FIG. 5. Toad 85 mm long. Captured and fixed 28 November 1974. Gland of a male during amplexus, which is smaller than those of animals caught earlier. Central lumen narrow with no coagulum. Few peripheral lumina, mostly empty. The number of parafollicular cells has increased.



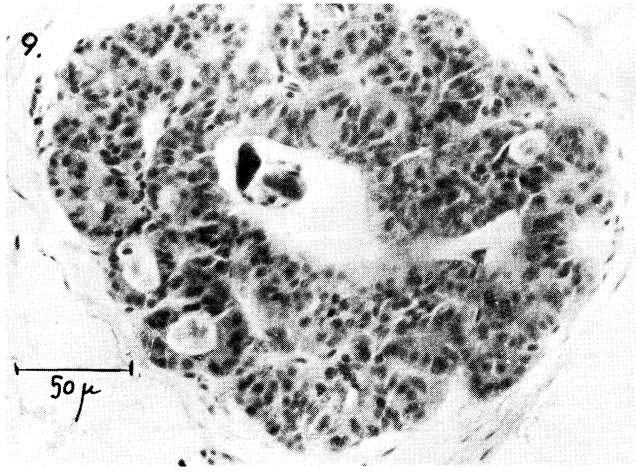


FIG. 6. Toad 80 mm long. Female in amplexus with male of Fig. 5. Gland slightly larger than that of male. No central lumen. Quite a few follicles with coagulum and many parafollicular cells.

FIG. 7. Toad 75 mm long. Captured and fixed 7 May 1969. Parafollicular cells and follicles rather evenly dispersed throughout the gland. Coagulum stored in some follicles.

FIG. 8. Toad 71 mm long. Captured and fixed 12 May 1970. Gland excised from an animal of the same season as that of Fig. 7, but a year later. Similar histological structure; less follicles and more parafollicular cells.

FIG. 9. Toad 80 mm long. Captured and fixed 14 June 1970. Little signs of activity despite the unusually persistent central lumen and coagulum in some peripheral follicles. This precedes the state of rest seen in Fig. 1.

DISCUSSION

The volume of the ultimobranchial body and the relative amounts of its components—follicles, parafollicular cells, connective tissue and capsule—change during the year. In June–July there is almost a total absence of follicles and coagulum. The rather large parafollicular cells—probably corresponding to ovoid (stem) cells (Robertson, 1971), which later transform into follicular cells—indicates a state of rest; there is no calcitonin (serum calcium-lowering hormone) produced, released or stored.

A surprisingly similar histological picture is recognized after hypophysectomy in young adults (Boschwitz, 1960b), or injection of calcitonin into adult toads (Boschwitz, 1973). After hypophysectomy, presumably a substance which normally maintains and activates the UB is removed, leading to involution of the gland.

The ultimobranchial body manufactures and secretes calcitonin in response to an elevated plasma calcium concentration. Calcitonin injections prevent this elevation and thus reduce or suppress the glandular hormonal production. This lack of stimulus finally results in the degeneration of the secretory cells.

The similar histology shown by the ultimobranchial bodies, either suppressed after exogenous administration of calcitonin or hypophysectomy to the gland of an intact toad in June–July, suggests that similar factors may be involved. In June–July normally the UB is at rest, presumably because of the reduced influence of the hypophysis; thus no calcitonin is produced.

The later development of a central lumen, of peripheral follicles and, finally, of coagulum secretion until December–January, reveals structural differentiation and renewed calcitonin formation, secretion and perhaps storage. The gland reaches its maximum volume, similar to the ultimobranchial bodies of toads injected with prolactin and of young adults after thyroidectomy (Boschwitz, 1960b, 1969), a result which shows that under certain circumstances prolactin and thyroxine are antagonistic (Etkin and Gona, 1967a, b). It also points to the possibility that prolactin either wholly or partly is the UB-activating principle of the hypophysis. If so, the involution of the UB after hypophysectomy would be due to prolactin deficiency.

The effects of hypophysectomy, of administration of the hypophyseal hormone prolactin, and, indirectly, also of thyroidectomy, are evidence that the calciostat is not independent of the hypophysis (see Salzer, 1971; Robertson, 1971; Boschwitz, 1960b).

The fact that the UB undergoes an annual cycle suggests that structure and function are not independent of, but rather related to, the reproductive cycle. The fact that peak lumen information and coagulum production occur in early spring at the time of the water drive to spawning sites, suggests an interdependence on environmental day-length, rainfall, humidity and temperature. It may be surmised that these factors influence the UB via the hypophysis, which is known to respond to changing photo-periods and temperature. A case in point is the temperature-dependent activity of the hypophysial prolactin, the potency of which increases with rising temperature in spring (Stevens, 1973) and which contributes to the water drive accompanied by change of skin texture in *Triturus viridescens* (Chadwick, 1941) and probably in *Bufo viridis* (Boschwitz, 1969). The effect of prolactin on the UB suggests that this gland is involved in the seasonal water drive (Boschwitz, 1969).

It may be assumed that the UB changes, concurrent with the water drive and amplexus, are due to osmoregulatory processes in which calcium metabolism plays an essential role.

Since the UB undergoes seasonal changes, production and secretion of its hormone calcitonin may also be variable. Serum calcium fluctuates seasonally in *Xenopus* (Zwarenstein and Shapiro, 1933) and *Bufo boreas* (Boschwitz and Bern, 1971). At certain times glandular activity may be heightened to maintain the calcium level near the annual mean circulatory level of about 10 mg/100 ml.

The appearance of the UB from the male and female specimens in amplexus is of special interest. The gland though in a state of storage, has capillaries in the vicinity of the follicles, which suggests that if secretion persists, as in other toads captured when not in amplexus, that it can be transported through the circulation. However, the precise stage when hormone is secreted into the circulation is not known. It may coincide with coagulum formation or with the following period of storage or with both.

Toads captured at the same place and time do not always possess similar ultimo-branchial bodies, a feature also recognised in the ovaries (C. B. Jørgensen, personal communication). As the environmental conditions are presumably practically the same then it is likely that endogenous inhibitory factors exist.

Does calcium influence the water drive? This question arises because the UB (and the parathyroids which contribute to the regulation of the calcium balance), is most developed in the terrestrial *Bufo viridis* and much less in *Rana ridibunda* and *Hyla arborea* (Boschwitz, 1960). The UB is vestigial or even absent in aquatic species like *Xenopus, Ambystoma* and *Cryptobranchus* (Robertson, 1971). As the UB is best developed at the time of the water drive, the shift from the terrestrial to the aquatic period and/or the immersion in water during amplexus seem to require a change in calcium balance.

The lengthening of the daily photoperiod and the rise of temperature increase the supply of food which contains calcium; they also induce the hypophysial secretion of gonadotrophins and prolactin, and indirectly oestrogen, which influence the plasma calcium level (Simkiss, 1960; Munday, 1968; Copp, 1970). Although the pathways of these homeostatic mechanisms are still not understood, it cannot be excluded that the seasonal UB changes are a consequence of the changing level of serum calcium.

Further research is required to examine the hypothesis that "external factors stimulate the hypophysis to increase its prolactin secretion, which influences the ultimobranchial body, the skin and the water drive (Boschwitz, 1969), probably by changing osmoregulatory mechanisms". The endocrine changes, though retained to some extent, are not seasonally correlated in captive animals. Captivity distorts the environmental changes normally experienced by specimens throughout the year. It obliterates the light-induced activation of the hypothalamo–hypophysial axis and consequently its effects on target organs (Boschwitz, 1967). Clearly the plasma calcium level should be determined throughout the year and co-ordinated with the histological structure of the UB, and such a study is being done.

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REPRODUCTION IN THE EASTERN DIAMONDBACK RATTLESNAKE, *CROTALUS ADAMANTEUS* IN CAPTIVITY, WITH COMMENTS REGARDING A TERATOID BIRTH ANOMALY

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SUMMARY

Reproduction in *Crotalus adamanteus* is documented and observations concerning sexual maturity, date of copulation, gestation period, date of birth and variation of the young are presented. A case of *duplicitas anterias* is described. A review of duplicity in rattlesnakes and comparisons with the bicephalic *C. adamanteus* are undertaken.

INTRODUCTION

Although wild-caught gravid specimens of *C. adamanteus* have given birth in captivity, information concerning specific elements of the reproductive cycle, namely sexual maturity, dates of copulation, gestation periods, dates of birth and variation of the young is fragmentary.

Taxonomically and geographically, the phenomenon of abnormal multiplication of parts is widely distributed. Accounts of axial bifurcation in ophidians, including rattlesnakes, have been reported in popular publications and scientific literature. In the belief that any additional data is valuable, we report on reproduction in captive specimens of *C. adamanteus* and describe a case of *duplicitas anterias* which resulted from this mating.

MATERIALS AND METHODS

A young male *C. adamanteus* measuring approximately 50 cm total length was obtained from an unknown locality on 14 June 1966. A female, approximately the same size and collected in the vicinity of Gainesville, Florida, was received on 8 October 1967. The snakes were maintained in glass-fronted fibreglass cages measuring 80 × 50 × 35 cm with paper substrata. Fresh water was provided daily and the snakes were fed freshly-killed laboratory mice and rats. The ambient temperature ranged between 27 and 35°C, and the relative humidity averaged 50%. Skylights provided a natural photoperiod.

OBSERVATIONS

On 15 January 1971, the male measured about 135 cm (total length) and weighed about 3.5 kg, and the female measured about 110 cm and weighed about 1.1 kg. Precise data were not obtained for fear of stressing the snakes. The snakes were placed together at that time and the female immediately coiled tightly with her head and tail beneath the coils. When the male touched her body, she responded with strong lateral flexures and this reaction persisted for several weeks. The snakes were separated shortly thereafter. The rattlesnakes were again introduced on 15 January 1972 but no copulation was observed and the male was removed a few weeks later. The snakes were maintained separately until 15 January 1973. On 30 January, coitus was observed at 0800 hr and they remained joined until 1700. Occasional pulsations near the cloaca of the male were seen by us but the female remained passive. On 1 August 1973 (a gestation period of 213 days), the female gave birth to one bicephalous (Figs. 1 and 2) and nine normally-formed young. The neonates ruptured the fetal membranes by thrusting upward, usually within 30 min of birth. One of the normal young was fully formed but dead at birth. The young were measured and weighed immediately, and the range of variation of the normal young was as follows: total length 30–38 mm, mean 33; weight 35.0–48.5 g, mean 43.2. Within a few weeks, the young accepted small laboratory mice (7.5 g).

The bicephalous snake survived for 12 h and the heads moved independently of each other. When forward movement was attempted, the right head initiated it, even though the effort was unsuccessful due to the severe contortions of the body. Both heads gaped, protruded the glottides, and shuddered convulsively in apparent attempts to breathe. The snake had somewhat active tongues in each head. The left head ceased moving approximately nine hours after birth. A radiograph was taken of the bicephalous specimen *post mortem* (Fig. 3). The snake was fused at the midline with pronounced bends in the body axis. The right-hand free anterior portion was 6 cm and the left-hand free anterior portion was 5 cm. Forty vertebrae were counted on the right-hand anterior portion and 36 on the left-hand portion. A common investment was formed by the unity of the integument of the two anterior portions. It was difficult to separate the posterior portions to measure them accurately. The overall body length was also difficult to measure because the snake was severely contorted but we estimate the length at approximately 15 cm. There was a bifid digestive tract that was fused only at the posterior portion, approximately 3 cm anterior to the cloaca. The overall length of the fixed, dissected, right digestive tract was approximately 20 cm while that of the left was approximately 17 cm. The right-hand portion had a relatively complete cardiovascular and respiratory system with both lungs and heart easily identifiable. The portion on the left was much more rudimentary but vestigial heart and lung were identified. The tracheae were normal. Kidneys, liver, spleen and pancreas could not be identified grossly or microscopically on either side. The jaws and mouths of both sides were well developed and capable of functioning. The fang mechanisms appeared normal. The head scalation and length and breadth of the right head was as follows with the right side first: supralabials 14 + 15; infralabials 18 + 18; canthals 2 + 2; scales between anterior canthals 2, scales between posterior canthals 4, total scales in prefrontal region 11; width 15 mm; length 24 mm. The left head was as follows: supralabials 14 + 15; infralabials 18 + 17; canthals 2 + 2; scales between anterior canthals 2, scales between posterior canthals 4, total scales in prefrontal region 13; width 14 mm; length 24 mm.

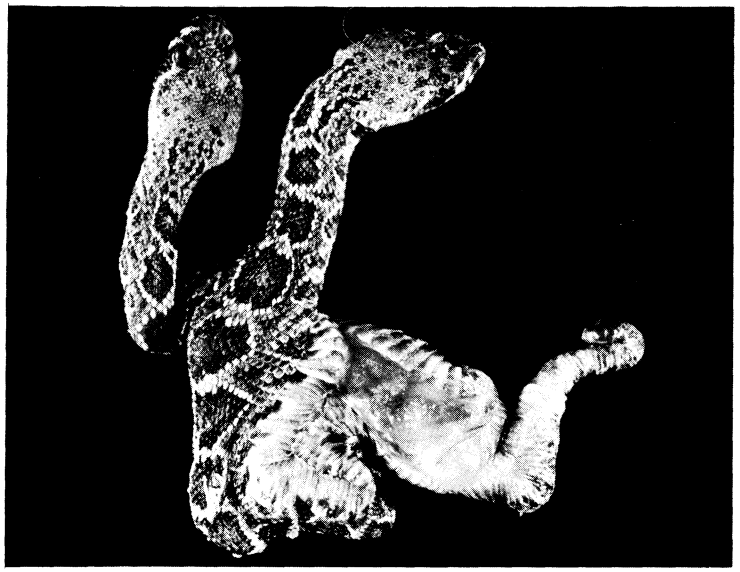

FIG. 1. Dorsal view of bicephalic *Crotalus adamanteus*.

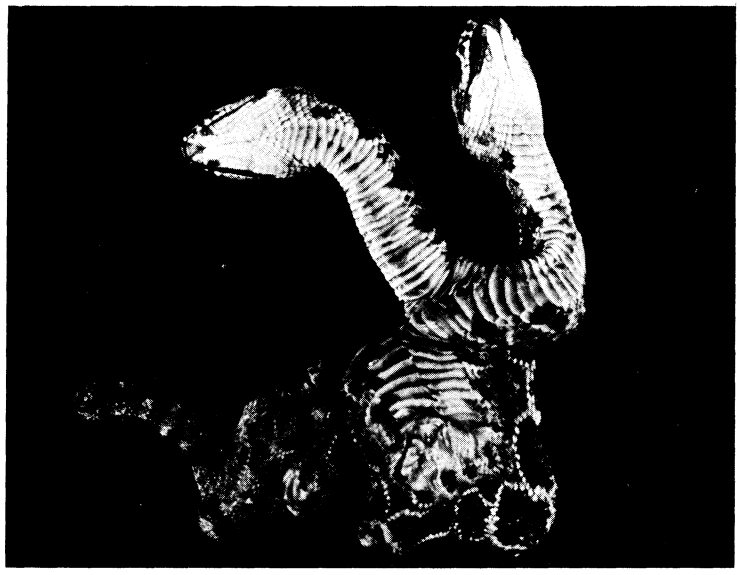

FIG. 2. Ventral view of bicephalic *C. adamanteus*.

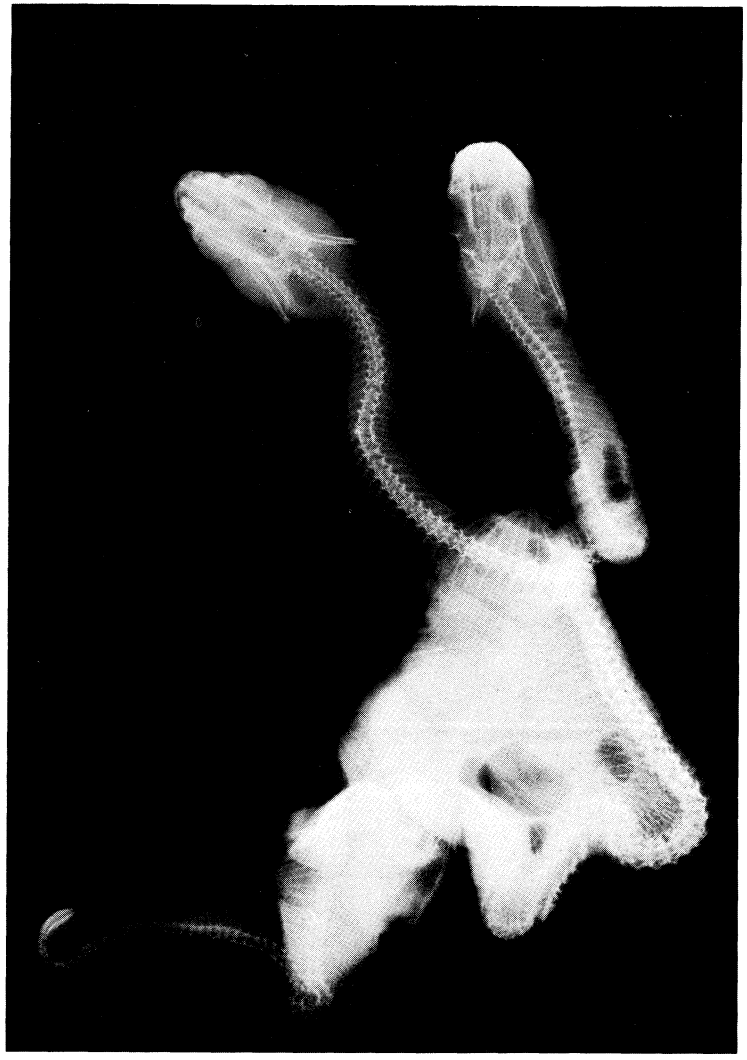

FIG. 3. Ventral-dorsal radiograph of bicephalic *C. adamanteus*.

DISCUSSION

The reproductive cycle of *C. adamanteus* is poorly known. Meek (1946) stated that this species mates in mid-September. Klauber (1956) gave parturition dates of 16 July; 25, 26 August; 5, 11, 14, 28, 29 September and 5 October. Klauber (1936) listed one brood of 18 and gave the range of young per brood as 8–21 (Klauber, 1956).

Cunningham (1937), in his analysis of axial duplication, divided the classification of bicephalic snakes into two main divisions: indeterminates and determinates. The indeterminate reports are so listed because the information available is meagre and some bicephalic rattlesnakes can be listed in this category. Baird (1856, cited by Klauber, 1956) reported on a two-headed rattlesnake which was collected at Camp Yuma, California, and there is a possibility that the bicephalic rattlesnake reported by Michler (1857) was the same specimen (Klauber, 1956). Anon. (1877) reported that a large rattlesnake from Breathitt County, Pennsylvania with two well-developed heads and necks was found but Klauber (1956) felt that the report might be doubtful. A juvenile bicephalic rattlesnake was found at Greenwood, Jackson County, Missouri (Levering, 1878). A bicephalic rattlesnake, approximately half-grown, was reportedly collected four miles west of Broken Bow, McCurtain County, Oklahoma (Klauber, 1956). The specimen was said to have two distinct heads which were independent with two tongues and four eyes.

The determinate classification (Cunningham, 1937) refers to those snakes where the description of the extent of duplication is more complete. Cephalic dichotomy includes specimens which have marked duplication with a single body and two heads fused or slightly separated. Anterior dichotomy includes those snakes which have marked duplication at the anterior end of the body. Posterior dichotomy refers to those specimens with the anterior part of the body single and posterior part of the body doubled. Amphi-dichotomy is the group where both anterior and posterior duplication occurs.

Cephalic dichotomy. Those snakes with part of the body doubled and the cranium bifurcated were called teratodymus, opodymus (Nakamura, 1938) and the following snakes are examples of this classification. Wiley (1930) found a bicephalic *C. b. basiliscus* that was joined at the eyes. There was one trachea and the tongues extended from a single opening. She wrote, the under parts were arranged in two almost perfect jaws, shorter on the inside and having apparently but two jaw bones, these being on the outside. Cunningham (1937) received a two-headed timber rattlesnake from R. L. Ditmars which was joined at the eye-level. Two eyes were in evidence and the head was broad. Klauber (1956) obtained a young *C. adamanteus* which had two heads joined at the eyes. One head was imperfect. Amaral (1926) found a young *C. d. terrificus* which was joined at the last supralabials. This type of duplication is *Cryptoderodimi: Iniodimi* (Belluomini, 1965). Klauber (1956) reported on a specimen of *C. v. oreganus*, a female less than ten days old, which was fused just behind the angles of the mouths. The specimen differed in head scalation between the two heads.

Rimkus (1947) photographed a specimen of *C. h. horridus* found near Logan, West Virginia, which was fused posterior to the angle of the mouth and was said to have the use of both tongues (Klauber, 1956). Kelly (1909) found a two-headed rattlesnake which had the heads joined at the angles of the jaws; Klauber (1956) felt that this specimen was *C. s. scutulatus*. Hyde (1925) listed two rattlesnakes with cephalic duplication. The first snake had the basal plates joined; one head had functional eyes and tongue whereas the other had no visible tongue and the mouth was sealed. The second snake possessed well developed eyes and tongues. R. Goellner (pers. comm.) observed a newborn bicephalic *Sistrurus m. streckeri* collected in the vicinity of Piedmont, Wayne County, Missouri. The snake was severely debilitated. The point of bifurcation was just posterior to the junction of the jaws. Both jaws and tongues functioned normally and the snake extended the fangs in the right head. The snake assumed a resting coil, vibrated its tail but crawled with great difficulty.

A bicephalic *C. atrox*, probably a few weeks old from New Braunfels, Texas was fused posterior to the junction of the jaw (J. Laszlo, pers. comm.). The jaws and tongues functioned normally. The snake lived for seven weeks and was said to have digested and defecated normally. The right head was dominant. Examination of a radiograph revealed that this specimen should be classified as *Crypto-derodimi: Iniodimi* (Belluomini, 1965). Lewin (1962) described a two-headed "swamp rattlesnake" from Wisconsin that was strongly bifurcated.

Anterior dichotomy. The second division of axial duplication recognised by Cunningham (1937) was Anterior dichotomy, and Nakamura (1938) classified this division as teratodymus, derodymus. Cunningham (1937) received a rattlesnake with the point of bifurcation 38 mm posterior to the bases of the skulls. Vanzolini (1947) studied a 150 mm specimen of *C. d. terrificus* which had marked division of the heads. One head had a complete neck. McMullin (1963) described a *C. v. viridis* with a single button from the vicinity of Glendive, Montana which measured about 265 mm with two necks 75 mm long. Both heads were functional. Klauber (1956) stated, the bicephalous snake with two distinct and perfectly formed heads is a rarity among rarities. Our specimen appears to have one of the most marked degrees of anterior cephalic division heretofor reported for rattlesnakes.

Amphi-dichotomy. An unusual *C. v. viridis* received by Klauber (1956) had the heads and necks separated for about 75 mm, then a single trunk for about 100 mm, with a 75 mm separation of the tail. This specimen would be classified as Amphi-dichotomous (Cunningham, 1937) and teratopagus, anakatamesodidymus (Nakamura, 1938).

The bicephalous specimen has been deposited in the vertebrate collection of the University of Texas at Arlington (UTA R-5545).

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 735–739

PARASITOLOGICAL TECHNIQUES IN HERPETOLOGY: RELAXATION AND FIXATION OF PARASITIC METAZOANS

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(*Received* 14 *June* 1976)

INTRODUCTION

This paper presents a number of established techniques that result in the correct relaxation and fixation of the metazoan parasites of reptiles and amphibians for taxonomic study. Herpetologists often seek information about parasites they encounter, yet seldom have reliable techniques available.

Reichenbach-Klinke and Elkan (1965) describe the killing of, and the dissection and fixation of the tissues from ectothermic vertebrates for routine pathological study. Kaplan (1968) describes the methods for sampling the body fluids, excreta and vital products of ectotherms and notes that no methods for examining faeces of ectotherms for parasites have been developed. He suggests that the techniques applicable to mammalian faeces should be utilised. The Neuchatel proceedings (1957) provide general information on the collection of parasites of vertebrates.

Techniques used in the preparation of parasitic material from reptiles and amphibians are scattered in various texts and journals. It is important to stress correct relaxation and fixation of many parasites. Only the techniques applicable to the parasitic Metazoa are considered here, though their use is not limited to the parasites of reptiles and amphibians. Instructions for the preparation of many required chemicals are provided in the Appendix.

TECHNIQUES

Certain larval spirometrid tapeworms (Cestoda, Pseudophyllidea) found within the tissues of reptiles and amphibians may infect man (Van der Hoeden, 1964). Likewise, Cooper (1974) pointed out the danger to humans from infection by pentastomes (Pentastomida) when dealing with infected reptiles. During, and directly following, the dissection of reptiles and amphibians, attention must be paid to routine hygiene.

Ectoparasites can be removed with little discomfort to the live host. Endoparasites are usually seen only on dissection. In live reptiles and amphibians, faecal examination aids the diagnosis of certain parasitic infections. Faeces may be fixed in hot 10% formalin and later stored in 70% ethyl alcohol (Soulsby, 1965). For diagnostic purposes, however, fresh faeces are far superior to preserved.

After death, reptiles and amphibians should be dissected or preserved forthwith, to prevent post-mortem effects masking parasitological examination. An alternative to prompt dissection is deep freezing of the fresh cadaver, for examination at a later date. Freezing restricts microbiological investigations and histological clarity, but serves to uniformly relax metazoan parasites. Subsequent identification of parasites is certainly

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possible, though some ectoparasites may be lost during freezing and thawing. Individual frozen host specimens should be stored in separate, sealed, clearly labelled polythene bags. The immersion of whole animal hosts in chemical fixative is not recommended if routine information on parasite taxonomy is required. Cysts or tumours of doubtful nature may be fixed fresh in 10% formalin.

Prior to relaxation and/or fixation, parasites may have to be freed from surrounding mucus and sometimes separated from host tissue. The removal of mucus is achieved by gently shaking the parasite in a small quantity of cold tapwater or saline. Some adult Cestoda, Acanthocephala and Acarina may be firmly attached to adjacent host tissue. Detachment, though desirable, should not result in damage to the parasite attachment organs, many of which are of importance taxonomically. The fixation of the parasite, along with a small piece of adherent tissue, is an alternative.

Individual parasite groups require special treatment, and the use of ethyl alcohol as a general fixative is best avoided. Fixation is usually achieved in an alternative fixative, but may be followed by storage in 70% alcohol.

Monogenea

After removal of excess mucus, they should be relaxed in cold tapwater (30 min to several hours) until they fail to respond to tactile stimuli. Monogeneans can be fixed and stored in either 10% formalin or alcohol–formol–acetic (A.F.A.).

Cestoda

Larval and adult Cestoda may parasitise reptiles and amphibians. Larval forms may be free or encapsulated. Using mounted needles, encapsulated larvae should be removed from their capsules, prior to relaxation and fixation. The scolex of some adult tapeworms may require careful separation from host intestinal tissues.

Live larval and adult cestodes must be relaxed in cold tapwater; complete relaxation may take from 2 to 24 hr. 5–10% formalin is the recommended fixative, since fixatives containing alcohol cause excessive hardening of tapeworm tissues (Schnur, 1969).

Digenea

Larval and adult digenetic trematodes occur within the bodies of reptiles and amphibians. Before relaxation, encysted larvae should be excysted by light pressure beneath a coverslip.

Digenea are killed by plunging into hot (60°C) water (Slusarski, 1958). This extends the parasite, a feature that permits examination of internal structures. Fixation is achieved in 10% formalin, or preferably A.F.A. Digenea may be stored indefinitely in A.F.A. However, these fixatives may distort, or even destroy, some of the internal structures of larval digeneans. Relaxation of larval *Diplostomulum* (*Tylodelphylus*) *xenopodis* (from *Xenopus laevis*) in cold saline, followed by fixation in ice cold 10% buffered formalin, was found satisfactory (Tinsley and Sweeting, 1974). Examination of live material is important in the detailed taxonomic study of larval Digenea.

Nematoda

For taxonomic purposes, parasitic nematodes can be prepared using either of the following methods. Live nematodes may be killed in hot 70% ethyl alcohol, and stored in cold alcohol. However, placing live nematodes in cold glacial acetic acid also kills them in the desired extended position (Berland, 1961). This method is particularly

useful under field conditions, when hot alcohol is unavailable. On return to the laboratory, nematodes killed in acetic acid should be transferred to cold 70% ethyl alcohol.

Acanthocephala

Acanthocephalans are often firmly attached to the host intestinal wall. Careful dissection is required to avoid damage to their spiny proboscis, and live parasites must be relaxed in cold water to extend this important taxonomic feature. Once the acanthocephalan fails to withdraw its proboscis when touched, it is fixed and stored in cold A.F.A.

Hirudinea

Leeches are capable of powerful muscular contraction and require careful relaxation. The leech is placed in a half-filled beaker of water, plus added quantities of 70% ethyl alcohol. The beaker should be eventually filled over a period of about 30 minutes. Gradual increase in alcohol concentration relaxes the parasite, and this is followed by fixation in 5% formalin and storage in 70% ethyl alcohol. The leech is maintained in an extended position by light pressure beneath a microscope slide, as the fixative is added (see Cox, Dales, Green, Morton, Nichols and Wakelin, 1969).

Mahoney (1966) recommends the use of 7% magnesium chloride as an anaesthetic for leeches, and Richardson (1975) found an ether/water mixture satisfactory in certain cases.

Arthropoda

Several groups of arthropods parasitise reptiles and amphibians. They are usually killed by direct immersion in the recommended fixative.

Mites and ticks (Acarina) can be killed and preserved in 70–90% ethyl alcohol, though Oudemann's fluid is preferred (Mahoney, 1966). This contains alcohol, plus a little glycerol and acetic acid which keep the limbs supple and aid penetration. When removing ticks, it is important to ensure that the hypostome is completely recovered. Failure to do this may result in an infection of the host at the point of attachment. A drop of methylated spirits or ether on these acarines, prior to their removal with forceps, is usually successful. Larval Diptera can be fixed and stored in 70% ethyl alcohol containing a few drops of glycerol (Mahoney, 1966). Pentastomida may be fixed in 6–8% formalin or formalin–acetic–alcohol (F.A.A.) (Self and Kuntz, 1957). If the latter fixative is used, the parasite is first killed in hot water.

Preserved parasite material should be despatched immediately to a parasitologist. Packing should preclude breakage or leakage and enclose data, a duplicate set of which should be kept by the sender. Information should include: mode of preparation of the parasite(s); host species; site of infection; host length, weight, sex and age (if known), and stomach contents (if any). Information on the country/locality of origin, time in captivity, date of death, conditions of maintenance, other reptile and amphibian species present and any recent unusual behaviour, are all vital. Whenever possible total parasite loads, rather than samples, should be collected.

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APPENDIX

Formalin

5%— 5 parts 40% commercial formaldehyde to 95 parts distilled water.
10%—10 parts 40% commercial formaldehyde to 90 parts distilled water.

10% (neutral) buffered Formaldehyde solution

40% commercial formaldehyde	100 ml
Distilled water	900 ml
Sodium dihydrogen phosphate (monohydrated)	4.0 g
Disodium hydrogen phosphate (anhydrous)	6.5 g

(N.B. The salts should be dissolved in a little warm water and then added to the rest of the water and formalin.)

Alcohol–formol–acetic (*A.F.A.*)

Absolute ethyl alcohol	720 ml
40% commercial formaldehyde	100 ml
Glacial acetic acid	50 ml
Distilled water	130 ml

Oudemann's solution (*simplified*)

70% ethyl ethyl alcohol	22 parts
Glycerol	1 part
Glacial acetic acid	2 parts

Formalin–acetic—alcohol (*F.A.A.*)

Absolute ethyl alcohol	900 ml
Glacial acetic acid	50 ml
40% commercial formaldehyde	50 ml

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BURROWING IN THE GREEN TOAD, *BUFO VIRIDIS*

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(*Received* 27 *June* 1975)

INTRODUCTION

Burrowing is well-known among the spade-foot toads, but has been little studied among the bufonids. Terentev and Chernov (1949) record *Bufo viridis* living in self-dug burrows up to 30 cm long in soft ground in the U.S.S.R. Smith (1951) states that *B. calamita* adults generally live alone in self-dug burrows, that in loose sand they use their hind legs to dig but in firm sand they use their forelegs, kicking out the sand like a terrier with the hind legs. Hazelwood (1974) found in Australia, that in sandy soil *B. marinus* made self-dug burrows. In Tunisia the Hottentot fig, *Carpobrotus edulis*, is used to stabilise artificial sand dykes. A series of such dykes which bordered a beach near Hammamet were examined in December 1972. Burrows of the green toad, *B. viridis*, occurred at intervals of about 10 m. The burrows, passing obliquely into the dyke, were inhabited by up to 12 toads. One burrow was 100 cm long and bifurcated into two chambers.

Several dozen of these toads were captured in order to observe their behaviour in captivity.

BURROWING IN CAPTIVITY

Captured toads were induced to burrow by keeping them on damp sand and illuminating the sand with a photo-flood lamp. Individual toads would excavate a shallow pit, using the typical backward digging movement that is seen in the spade-foots. The toad would then turn round and use its front feet to dig and kick out the sand with the hind feet.

The facilities provided for this experiment permitted toads to burrow in this fashion for up to 20 cm. The relative pad sizes of the forefeet of *B. viridis* were compared with those of *B. marinus, B. woodhousei, B. bufo, B. calamita, B. carens, B. kiririnyagae* and *B. regularis*. There was no obvious difference in the size and shape of the front feet of *B. viridis* and the other toads.

CHOICE EXPERIMENTS

Twenty captive toads were used. It was found that when offered opaque plastic tubes 15 cm long and 5 cm wide they would hide in these during the daytime. When single toads were given a choice of sitting in one of four tubes it was found that they chose the tubes at random (Table I). They were then tested for their choice of tubes in groups of four, and the results of twenty such trials are summarised in Table II. The % expected frequency was calculated from the 4^4 possible permutations assuming no interaction between toads. The Chi^2 value of 2940.8 is highly significant. It can be seen

TABLE I. Random choice of tubes

Tube No.	Number of toads choosing
1	7
2	6
3	5
4	8

$Chi^2 = 0.77$, not significant.

TABLE II. Clumping by toads in groups of 4

Distribution of toads in 4 tubes	Frequency of occurrence of 20 tests	% Frequency of occurrence	Expected % frequency of occurrence
4 0 0 0	12	60	1.6
3 1 0 0	3	15	18.8
2 2 0 0	4	20	14.1
2 1 1 0	1	5	53.3
1 1 1 1	0	0	9.4

$Chi^2 = 2940.8$.

that there was distinct clumping. In only 5 out of 80 choices did toads occur singly, whereas if the choices were random 33 would have been expected to have occurred singly.

In another test, 20 toads were each given a choice between four plastic tubes which had been sawn in half in order that a wire barrier could be inserted in the middle. Two of the tubes were otherwise empty, and two contained moss. Out of the 20 tested, 16 toads chose tubes in which the chamber contained moss, and 4 chose empty tubes. The Chi^2 value of 7.2 is significant suggesting that toads selected tubes that contained moss. The experiment was repeated, but using a captive toad in the chamber instead of moss. Thirteen toads chose tubes which had a toad inside, and seven chose empty ones. Although twice as many toads chose tubes containing another toad, the Chi^2 value of 1.8 is not significant.

DISCUSSION

It would appear that forward burrowing among the bufonids is a behavioural potential, as no special physical adaptation is demonstrated, and the phenomenon probably occurs in a wide variety of *Bufo* species where there are soils of the right type.

In the case of *B. viridis* in Tunisia, there is the problem of whether the clumping together in the self-dug burrows is a social response. The experimental results are to some extent inconclusive. It was shown that the toads clumped together when they are in a group, but if they are given the choice of a tube in which they do not have bodily contact with the contents, and the tube contains moss, there is a positive choice for these tubes as well. In the case of the choice of moss it may be that they are selecting

holes of high humidity. But in the tests in which there is bodily contact there is a much stronger response, so it may in part be a combination of a social response and of seeking holes with high humidity.

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BREEDING OF THE SNAKE, *LAMPROPELTIS MEXICANA BLAIRI*, IN CAPTIVITY

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(*Received* 12 *November* 1977)

In July 1972, a male specimen of *Lampropeltis mexicana blairi* was purchased from an American dealer; a female was purchased a month later from the same supplier. Their previous history was unknown; the male was 66 cm long and the female slightly longer. Since then they have been kept together in a 61 × 31 × 38 cm vivarium, and fed on dead adult white mice (approximately one each per week). No sign of attempted cannibalism has been seen but as a precaution they were watched while feeding. The vivarium was heated with incandescent bulbs controlled by a time-switch (18 hr light: 6 hr dark). The temperature was 24–32°C during the light period and 18–21°C during the dark. The same photoperiod was applied throughout the year. Under this regime there were no signs of mating.

In January 1977, the photoperiod was changed to give a longer dark period (14 hr light: 10 hr dark) (see Wagner and Slemmer, 1976). On 13 April 1977, mating was observed; at this time the female was 85 cm long and the male 94 cm. No signs of aggression, biting for example, were seen during mating. The female sloughed on 19 May and laid four eggs (batch 1) on 29 May. All the eggs appeared normal, and the maximum and minimum dimensions were 47 × 19 mm and 39 × 19 mm respectively. The female continued to feed throughout this period except when sloughing.

On 17 June, mating was seen again and on 27 July the female laid four more eggs (batch 2). Of these, three appeared normal (40, 42 and 42 × 19 mm), while one was small and discoloured.

The eggs were incubated in a plastic box, 24 × 17 × 13 cm, with one pin hole in the top. The eggs were on paper towels (three layers) with one layer over the eggs but prevented from touching them by a wire frame. The paper was kept slightly damp but it did dry out for a a day or so. The temperature in general was 24–27°C but did change to as low as 21°C and as high as 31°C on occasions; the eggs were kept in darkness.

Of batch 1, the first egg started to split at 09.00 hr on 13 August (76 days of incubation) and the young snake had emerged by 17.00 hr. Two other eggs had hatched by 19 August. The fourth was hatched a week later after failing to hatch; it contained a fully-formed but dead snake with an apparent deformity half way along its back. Of batch 2, the first egg was split at 18.00 hr on 3 October (68 days) and the snake had emerged by 06.00 hr the following morning. The other good eggs hatched on 5 and 6 October.

The hatchlings were 24–25 cm long; five resembled the female in coloration being relatively light, the other is darker like the male. They first fed (on pink mice) on 27 August, 5 and 11 November (batch 1) and on 11 November (first of batch 2).

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BRITISH JOURNAL OF HERPETOLOGY, Vol. 5, pp. 747–753

LETTERS TO THE EDITOR

ANOTHER BLACK GRASS SNAKE

Halfpenny and Bellairs (*Br. J. Herpetol.*, **5**, 541, 1976) reported the finding of a black grass snake (*N. natrix helvetica*) in North Staffordshire in May 1975. Several years earlier, on 22 August 1971, I found a black grass snake, approximately three feet in length, to the north-west of Gloucester. Dorsally, the snake was entirely black while ventrally it was a smokey-grey colour with a series of black patches extending laterally. Unlike the specimen described by Halfpenny and Bellairs, in which the "collar" was a whitish band confined to the ventro-lateral surface of each side of the lower jaw, this specimen had the usual yellow collar of the typical form.

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Highnam
Gloucester
18 May 1977

CONSERVATION OF THE GHARIAL

In 1974 the Indian gharial (*Gavialis gangeticus*) was on the verge of extinction, and probably the rarest of the world's crocodilians (*FAO India: A preliminary survey of the prospects for crocodile farming 1974* (based on the work of H. R. Bustard). FAO Rome (FO: IND/71/033), October 1974). It is unique, being the only surviving member of the family Gavialidae. For this reason tremendous international interest has centred around the project of the Indian government, with FAO/UNDP technical assistance, for its conservation, using management techniques involving eggs, hatchlings and extended post-natal care developed by myself in Australia for both crocodilians and sea turtles.

This project, started only in 1975, now holds 1406 growing gharial, and operates in the Indian States of Rajasthan, Uttar Pradesh, Orissa and Andhra Pradesh as well as in the Kingdom of Nepal. This increase in population compares with about 60–70 in the wild in India when the project was started, and a similar or lesser number in Nepal.

Hatchlings are reared in captivity until they reach a length of 1 m at about eighteen months old, when they are released into specially-gazetted sanctuaries in areas of ideal habitat selected as a result of India-wide surveys by myself. At the time of writing there are four such sanctuaries in India and one in Nepal. All are large, viable areas, the largest being 750 km^2. When this letter appears several hundred gharial will have been released back into the wild, in the largest programme of its kind for any endangered crocodilian, and a further hatch of over 1000 babies will have occurred. The keen interest of the Government of India—who have recently extended the project for a further four years—has ensured that the gharial is now no longer in danger of extinction. During the project several thousand will be put back into well-protected sanctuaries and National Parks, and the gharial will no longer be endangered.

The project, which includes captive breeding, extends to the greatly depleted salt-water crocodile (*Crocodylus porosus*) and Indian mugger (*Crocodylus palustris*). Further details of this work and reprints can be obtained from me.

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AN ENGLISH COLONY OF THE ALPINE NEWT

Smith (1951) suggested that the alpine newt (*Triturus alpestris*) would succeed in Britain if introduced; he stated that a colony had existed in Surrey for many years. In April 1970, I released four male and three female *T. a. alpestris* into a garden pond near Market Drayton, Salop (Grid Reference SJ670340). Breeding was successful, and a number of newly-metamorphosed newts were found close to the pond.

The garden has four artificial ponds, three 2 m apart, the other 20 m away. In April 1974, breeding newts were in all four ponds, and on 24 April 1977, 14 males and 16 females were counted (others were probably overlooked in the aquatic vegetation). Many tadpoles were present on 25 September 1977 and 18 recently-metamorphosed young were found under adjacent stones.

A number of smooth newts (*T. v. vulgaris*) and a few great-crested newts (*T. c. cristatus*) also breed in these ponds. The garden is situated on the edge of a village, adjacent to open countryside, and the newts were exposed to natural predators. Predatory insects, for example *Dytiscus marginalis*, were present, and a grass snake (*Natrix n. helviticus*) has been seen on several occasions; there are many cats in the village. There were no fish in the ponds, which were designed to encourage the breeding of amphibians (Bell, A.P., Garden ponds—an aid to conservation, *Shropshire Conservation Trust Bulletin* 30, 1974).

These observations confirm the opinion of Smith (*The British Amphibians and Reptiles*, London: Collins, 1951) that the alpine newt should do well if introduced on a wider scale. From a relatively small number of individuals a colony has survived and increased, and has spread at least 20 m during a period of seven years. Further studies of other nearby ponds are necessary to assess the future spread of this population.

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30 September 1977

PARTIAL NEOTENY

Further to my paper, *Partial neoteny in an Australian frog*, which appeared in the Journal in 1958 (**2**(6)) I recorded another example in the same species (*Mixophyes fasciolatus*). In March 1957 I collected a number of tadpoles. One of these did not start to metamorphose until August 1963. Unfortunately, owing to a death in the family, I had to leave home and on my return in mid-September the young frog was dead; death at the time of metamorphosis appears to be very common.

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CAPTIVE BREEDING OF MEDITERRANEAN TORTOISES

Information on the captive breeding of Mediterranean tortoises is urgently required in the hope that this may help towards providing a basis for the development of a commercially-sound management and breeding technique to be linked with legislation on the bulk importation of these species (see *BHS Newsletter* 15, 1976 and 16, 1977). Preliminary reports suggest that *Testudo hermanni* breeds relatively more easily in Britain than *T. graeca*.

M. R. K. LAMBERT
c/o B.H.S.

BOOK REVIEWS

NORTH AMERICAN HERPETOLOGY. By John Edwards Holbrook. Facsimile (1976) edited by K. Adler. Published by Society for the Study of Amphibians and Reptiles, Morton Hall, Ohio University, Athens, Ohio 45701, U.S.A. Obtainable from Dr D. Taylor, Department of Zoology, Miami University, Oxford, Ohio 45056, U.S.A. $60.

Holbrook, who came from a prosperous American family, was born in South Carolina in 1796. After qualifying M.D. at the University of Pennsylvania in 1818, he visited Europe and probably developed his interest in herpetology in the Jardin des Plantes in Paris, where he met some of the leading biologists of the time, including Cuvier, Duméril, Bibron and Valenciennes. On his return to America he established a practice in Charleston, South Carolina and married Harriett Pinckney Rutledge, a member of two of the state's leading families. Indeed, some of her ancestors signed the Declaration of Independence and the Constitution. Holbrook was thus wealthy and independent; in fact he personally owned seven slaves among the thirty-three owned by his wife's family. Holbrook was also Professor of Anatomy at the Medical College of the State of South Carolina, though by all accounts he preferred not to perform any surgery.

During the 1820s he started work on *North American Herpetology*. Its preparation took about 14 years. The present facsimile edition is that of the original second edition published in 1842, which comprises volumes 1–5. The present edition includes as extras a frontispiece of a new portrait of Holbrook by David M. Dennis commissioned by the Society. There is also an introduction by the editor, an account of the life and work of Holbrook, the new genera and species described by Holbrook and a list of the current nomenclature of scientific names in Holbrook's work. There are two editions of the reprint; a regular edition at the price quoted and a patron's edition bound in a similar way to the original. A thousand copies have been printed.

The five volumes include the description, distribution, habits and general comments on a large number of species. Indeed, the work is probably the first major herpetological contribution of this kind to be produced by an American in America. All the species described are illustrated, mainly in black and white, and there are nineteen beautiful illustrations in colour. Some of the black and white illustrations are not particularly good but this is a minor criticism.

This is a most handsome book, and the work is of great classical interest. Many biologists will not be familiar with this work or the author. For those interested in the history of biology, especially of the United States, and also for those who enjoy first class descriptions, this will be a welcome edition. Those readers who love books and natural history will derive much enjoyment from turning the pages of this beautifully produced work. However, for this pleasure the price, of necessity, is high, and I expect that viewing will be in libraries rather than from one's own copy.

H. Fox

Reptiles and Amphibians of Australia. By Harold G. Cogger (1975). 584 pp. Sydney: A. H. and A. W. Reed (U.K. agents: Bailey Bros & Swinfen Ltd, Warner House, Folkestone, Kent). £21.65.

The preparation of this book, clearly a major work, written and illustrated by Dr Cogger, must have been a massive undertaking. As the jacket says, "... by providing, for the first time in nearly a century, identification keys to the entire Australian herpetofauna [more than 660 species]. These keys, together with nearly 800 photographs (192 in colour) of living reptiles and frogs, 664 distribution maps, line drawings, definitive text and selected references, combine to provide a unique and comprehensive guide to these important and fascinating animals".

For each family, keys lead to genera and then to species. For each species, there is a description, notes on distribution and habits, a distribution map and a reference to a figure in most cases. Before the main text, introductory notes cover description, names, figures, distribution, habits, subspecies, making an identification, conservation and protection, location of specimens, collecting methods, preservation of specimens, captive specimens and snakebite. The coloured illustrations are excellent and very well produced, while those in monochrome are clear but less valuable.

Of course, there will be arguments over taxonomy but the author seems to have adopted a commonsense attitude to this and other problems. My only complaint is that the bibliography is sparse.

For anybody dealing regularly with Australian reptiles and amphibians, I would have thought this book to be essential, and not too expensive considering its size and scope. Certainly, all herpetological libraries and visitors to Australia should have a copy.

M. Peaker

Fair Play for Frogs. By R. Waldie and N. J. Frobish (1977). 178 pp. New York: Harcourt Brace Jovanovich. $7.95.

Even when I reached p. 178 I was not sure whether I had been hoaxed right through or whether I should have taken seriously what I had read. If the dust cover (the most serious part of the book) is to be believed, Congressman Waldie in 1962 introduced a Bill designed to legalise the stunning of frogs by means of a catapult so that they could then be killed and eaten. To this legalisation of frog-murder Frobish objected, and pursued Mr Waldie for the next 15 years, by means of an unrelenting correspondence, to make him see the error of his ways and to recant. How their correspondence got into the hands of an editor, and how this editor managed to get the two to agree to publication, we are not told. However, on the back dust cover we are assured that these "coy, clever, sanctimonious, hortatory and threatening" letters have actually passed through the post, and we must, therefore, I suppose, take them seriously—if we can. If you have that kind of humour, read the book. It certainly is a unique publication.

E. Elkan

This Broken Archipelago: Cape Cod and the Islands, Amphibians and Reptiles. By J. D. Lazell, Jr. (1976). 260 pp. New York: Quadrangle/New York Times Book Co. £7.

Dr Lazell has produced an engrossing and amusing account of the rich herpetofauna of the Cape Cod Islands. All the species found in the Archipelago are described in detail and with numerous first-hand anecdotes. The enthusiast of North American reptiles and amphibians will, I am sure, enjoy this informal, relaxed book by a practical herpetologist with extensive local knowledge. The book is generously illustrated with distribution maps and monochrome photographs of not only all the listed species but also of habitats—a subject covered in great detail. However, many of the illustrations have suffered during production, being either too small or too dark to be of much value.

D. R. Blatchford

Reptiles. By John Foden and Michael Sutton (1976). 94 pp. Edinburgh and London: Bartholomew.

Tortoises. By David Robinson (1976). 94 pp. Edinburgh and London: Bartholomew.

These little books are an introduction to the husbandry of reptiles for pet-keepers. To deal with the texts first, that by Foden and Sutton is reasonably good; there is a lot of advice but some of it is unqualified. For example, young chicks are good food for bird-eating snakes but a number of authors have stressed the problems of using them exclusively or for long periods. Again, Robinson gives practical advice but his descriptions of species are sometimes incorrect or inappropriate. Many of the species described have not been imported for years, and there is the usual mix-up between *denticulata* and *carbonaria*, the latter not even being mentioned. Robinson's use of the words *turtle* and *terrapin* falls between English and American, and is bound to cause confusion.

In many ways the books are old-fashioned—updated versions of several that appeared on vivarium-keeping in the 1950s—because they seem to engender a

"collectors'" approach to keeping reptiles as a hobby. Little mention is made that everything in the reptilian garden is not lovely. I find nothing on the Dangerous Wild Animals Act in the section on crocodilians and no mention of import restrictions on many species. Surely what the newcomer should have is a guide to the husbandry of reptiles that will breed readily in captivity because amateur herpetologists do have a role in determining the conditions required for reproduction as part of an overall scheme to understand the general principles of reptilian husbandry and breeding.

Both texts are marred by numerous spelling errors, but both books are ruined by the illustrations which are quite the worst I have ever seen. Not only are the coloured drawings horrid, but the legends are mis-spelt. According to one illustration, the false gavial lives where, as far as I know, the Chinese alligator lives, while the gavial proper lives nowhere at all! With the quality of illustrations and the lack of care in correcting the text, the publishers should be ashamed, because justice has not been done to the efforts made by the authors in producing a useful, if dated, text. Finally, no guides to further reading are included—a common but very serious omission in such introductory guides.

M. PEAKER

THE CARE OF DESERT REPTILES. By Karl H. Switak (undated). 26 pp. Published by K. H. Switak, 350 Molimo Drive, San Francisco 94127, U.S.A. $1.50.

This glossy, soft-backed booklet, written, illustrated and published by Switak, who is herpetologist at the Steinhart Aquarium, San Francisco, is a gem. As well as including a general guide to the principles involved, the care of eleven species of American lizard, one tortoise and nine snakes is described by an expert. The colour photographs of the reptiles and of the types of desert habitat found in the U.S.A. are superb. It is a book that every reptile keeper, amateur and professional, should buy, and one that he can afford.

M. PEAKER

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE

The required six months' notice is given of the possible use of plenary powers by the Commission in connection with the following names listed by case number (see *Bulletin of Zoological Nomenclature* **34** (part 1) 1 July 1977):

2135 Bonelli, 1811, "Tabula Synoptica": proposal to rule an available work.

2163 *Synapturanus* Carvalho, 1954 (Amphibia): designation of type-species.

Comments should be sent in duplicate (if possible within six months of the date of publication of this notice), citing case number to R. V. Melville, The Secretary, International Commission on Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, England. Those received early enough will be published in the *Bulletin of Zoological Nomenclature*.

The draft third edition of the International Code of Zoological Nomenclature is now available for comment by zoologists. Copies may be obtained (price £2.50 surface mail, £5.00 air mail) from the Publications Officer, International Trust for Zoological Nomenclature, c/o British Museum (Natural History), Cromwell Road, London SW7 5BD, U.K. Comments should be sent as soon as possible, and in any case before 30 November 1978, to the Secretary, International Commission on Zoological Nomenclature, at the above address.

A paper explaining the major changes proposed by the Commission's Editorial Committee to the existing Code has been published in the *Bulletin of Zoological Nomenclature* vol. 34, part 3. Copies may be obtained (price 50p) from the same address as copies of the draft Code.