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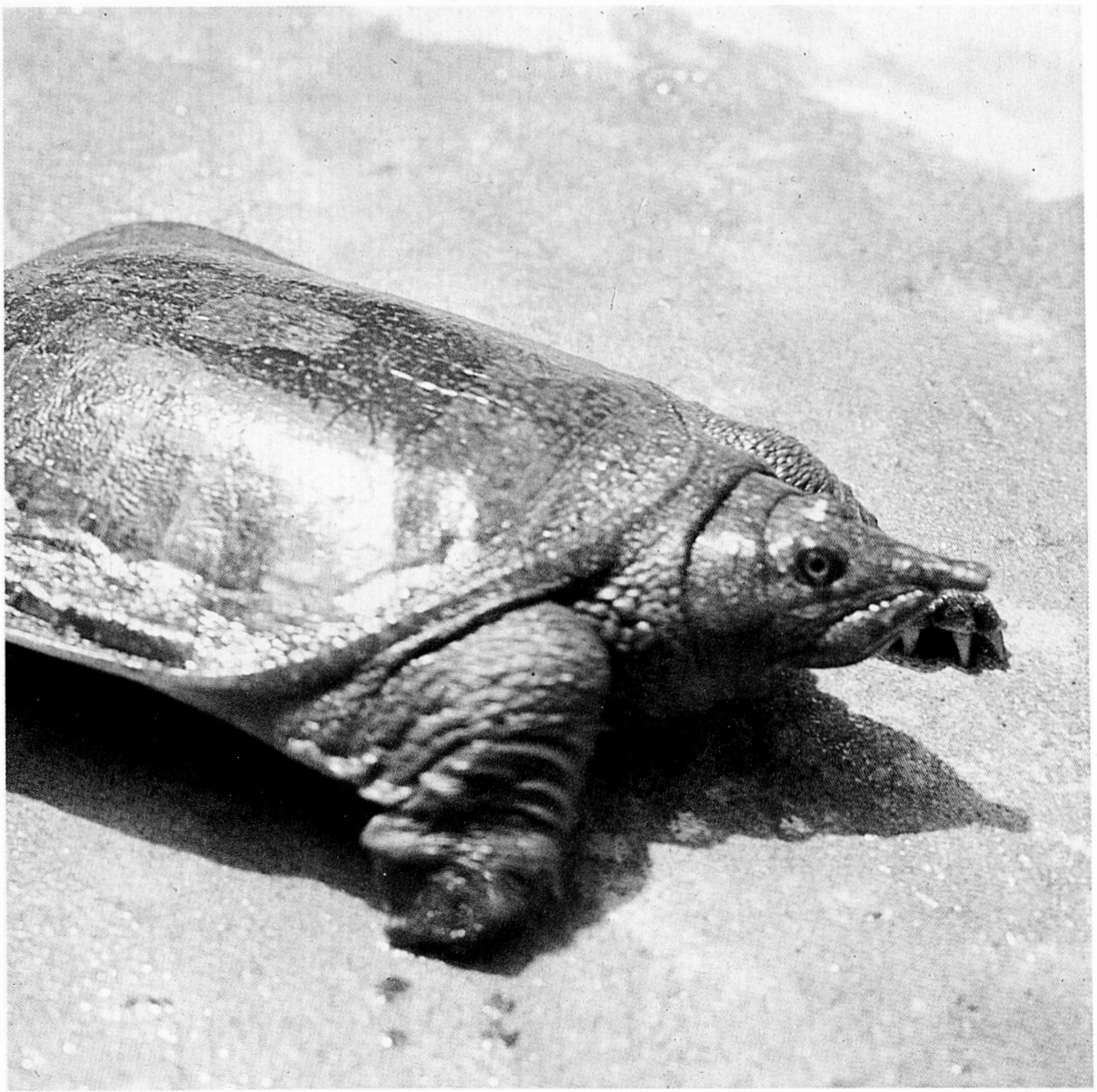

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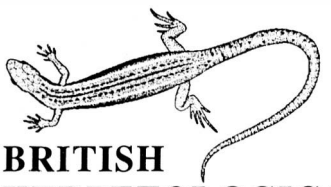

BRITISH HERPETOLOGICAL SOCIETY

FRONT COVER: Black-rayed softshell turtle, *Amyda cartilaginea* (Mark O'Shea)

VALIDITY OF THE MOUNTAIN GECKO *GYMNODACTYLUS WALLI* INGOLDBY, 1922

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(Accepted 13.3.91)

ABSTRACT

New material of *Gymnodactylus* from Chitral, Pakistan has been compared with that already in museums. *Gymnodactylus walli* which has been synonomized with *G. stoliczkai* by several authors was found to be a valid species. *G. walli* is redescribed, with notes on its habitat.

INTRODUCTION

Ingoldby (1922) described *Gymnodactylus walli* from a juvenile specimen (BMNH 1910.7.12.1.), collected from Drosh Fort, Chitral, North West Frontier Province (NWFP), Pakistan. Later Smith (1935) described *G. chitralensis* on the basis of two geckos collected from Karakal, Bumhoet Valley, Chitral. The types of both taxa are in the British Museum (Natural History), London.

In 1986, Mr K. J. Baig, Research Associate, Pakistan Museum of Natural History, Islamabad, Pakistan, collected a pair of geckos from Ghariet, a small village near Drosh Fort, Chitral. Both geckos were found to conform to *Gymnodactylus walli* and *G. chitralensis*. Later the type and syntype were received from the British Museum (Natural History), London, and a comparison confirmed that these geckos were conspecific, validating *G. walli* which has priority over *G. chitralensis*.

Ingoldby's account (1922) of the morphology of *Gymnodactylus walli* is brief and inadequate since it is based on a juvenile specimen. It has created ambiguity in the literature, so that *G. walli* was placed in synonymy of *G. stoliczkai* Steindachner, 1869 by almost all herpetologists (Smith, 1935; Minton, 1966; Mertens 1969; Khan & Mirza, 1977; Szczerbak & Golubev, 1986). Collection of the new material from Chitral, has made possible a redescription of *G.walli*. The following description is based on BMNH 1946.8.23.19.

The chaotic taxonomy of angular-toed geckos of the circum-Himalayan region has recently been augmented by further partitioning of them into several genera and subgenera (Szczerbak, 1986, 1988; Szczerbak & Golubev, 1984, 1986). I prefer to use the original generic designation for *Gymnodactylus walli*, *Gymnodactylus stoliczkai* and *Crytodactylus yarkandensis* Anderson 1872.

SPECIES DESCRIPTIONS

Gymnodactylus walli Ingoldby, 1922

Gymnodactylus walli Ingoldby, 1922. J. Bombay Nat. Hist. Soc., 28:1051

Gymnodactylus chitralensis Smith, 1935. Fauna British India, vol. 2, 46-47.

Tenuidactylus chitralensis Szczerbak and Golubev, 1986, Geckos of USSR and adjoining countries, 201.

Material examined. Holotype BMNH 1910.7.12.1., a juvenile collected from Drosh Fort, Chitral, NWFP, Pakistan, by Frank Wall; BMNH 1946.8.23.19. (Fig.1), an adult female, collected from Karakal, Bumhoet Valley, Chitral, NWFP, Pakistan; MSK 0484.86 and MSK 0485.86, both males, collected from Ghariet, Chitral, NWFP, Pakistan, by K. J. Baig, July, 1986.

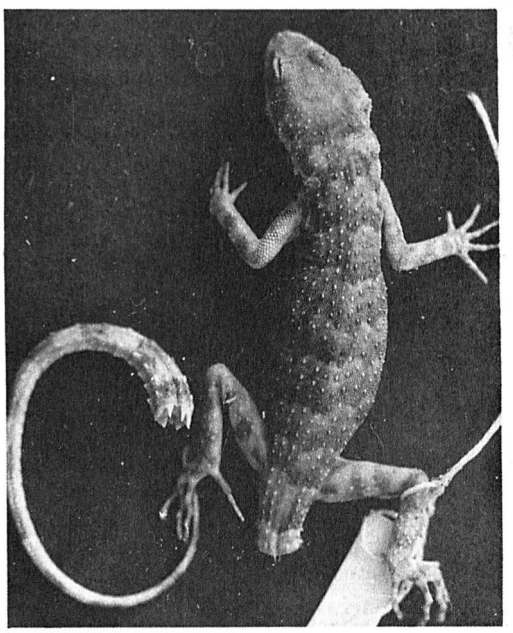

Fig. 1. *Gymnodactylus walli* Ingoldby, 1922, BMNH 1946.8.23.1., dorsal view.

Diagnosis. Medium sized geckos; tail longer than body; 20–21 interorbital scale rows; body dorsum with oval to round, nontridhedral, keel-less tubercles, arranged in 10–13 longitudinal rows, 21–23 in paravertebral row; 38–40 scale rows across midabdomen 160–170 scales along midventrum of body; 4–5 preanal pores in male.

Description of BMNH 1946.8.23.1. Habitus slightly depressed; rostral scale pentagonal; a patch of five postrostral large tuberculated scales; a pair of nasal scales on each side; few large tuberculated scales scattered on temporal region; 19–20 heterogenous interorbital scales; supralabials 11, progressively decreasing in size from first to last; 9–10 infralabials, first three equal in size; a prominent depression at postnarial, frontal and loreal region lined with distinctly smaller scales; body dorsum with 12 longitudinal rows of large oval tubercles which are 23–24 in a paravertebral row, those of four medial rows longer than broad, while laterals smaller and round, separated by 3–4 granular scales which are broader than long and arranged in irregular transverse rows, slightly imbricate, with lifted posterior ends.

Mental scale large, pentagonal, with acute angle, three pairs of postmentals on right and two on left side, first pair in contact by a suture length of which is one third the length of mental scale; 30–40 scales across midbelly which are hexagonal and strongly imbricate; 174–180 scales along midventrum of body from postmental to anterior of vent. No preanal or femoral pores, since BMNH 1946.8.23. is a female, instead there is a row of four large scales at the site of preanal pores, six oblique rows of large juxtaposed scales are anterior to vent.

When limbs are extended forward tips of toes hardly reach the axilla and fingers the snout tip; fourth finger with 20 and fourth toe with 23 subdigital lamellae, those on basal part much broader than those on compressed part of the digits, swollen at the angles of the digit, claws small fine and curved. Limb dorsum with large keel-less imbricate scales, several tubercles on thigh and shank, those on shank larger and slightly keeled; no postfemoral or subfemoral tubercles.

Tail longer than body, depressed, segmented which are more distinct at the basal part of the tail; dorsum of each caudal segment with 5–6 transverse rows of slightly keeled, heterogenous, imbricate granular scales with acute tips; three rows of large, slightly keeled tubercles on each side of the tail, six to a segment arising from its middle, becoming gradually imperceptible by the middle of the tail; subcaudals broader than long, two to a segment, however first two caudal segments have three subcaudals each, first subcaudeal of second and third segment fragmented into five and four smaller scales respectively.

Colour. Dorsum of formalin preserved specimens light grey with nine transverse brown wavy bands from posteriorum of nape to the level of vent. A dark stripe from eye joins the band on the nape. Limb dorsum barred. Tail with 13 dark and 12 light bars.

Measurements. Total length 129.9 mm; body length (SVL) 51.5 mm; tail length 78.4 mm; head length 13.1 mm; head breadth 9.5 mm; snout length 5.4 mm ; diameter of eye (not bony orbit) 3.25 mm; vertical diameter of ear opening 1.3 mm; length of oculo-oribital space 3.6 mm.

Variations. Table 1 quantifies differences in the morphology of specimens of *Gymnodactylus walli* examined. BMNH 1910.7.12.1., is a juvenile (Total length 57.65 mm), while BMNH 1946.8.23.1., is an adult female while MSK 0784.86 is

Character	1	2	3	4
Snout vent length (SVL)	51.5	26.65	54.7	46.7
Tail length	78.4	31	Br	52R
Supralabials	11/11	11/9	10	11/11
Infralabials	9/10	9/8	8	8
Interorbitals	21	20	20	21
Scales across midbelly	38	40	40	40
Dorsal tubercle row at midbody	13	10	12	12
Paravertebral row of tubercles	23	23	22	21
Lamellae under - fourth finger	20	20	20	19
Lamellae under - fourth toe	23	25	23	23
Midventral scale counts	174	169	162	160
Preanal pores	4(2–2) scales	4(2–2) scales	4	4
Postmentals	3/2	3/3	3/2	3/3
Head length	13.1	8.9	11.4	13.95
Head breadth	9.5	5.6	10.5	9.3
Head Height	5.75	3.9	5.5	5.5
Eye diameter	3.25	2.6	3.1	3.1
Ear diameter	1.3	2	1.5	1.5

TABLE 1. Variation in pholidosis and measurements (mm) of specimens of *Gymnodactylus walli* studied. (Br=broken; R=regenerated; 1=BMNH 1946.8.23.1.; 2=BMNH 1910.7.12.1.; 3=MSK 0484.86; 4=MSK 0485.86).

an adult male with regenerated tail, and MSK 0785.86 is a tailless adult male. Except for slight pholidosic variations, this series of geckos appear to be morphologically similar. MSK 0784.86 appears to be oldest of the series, as it is longer (SVL 54.7) and most robust. BMNH 1910.7.12.1 is morphologically abnormal at rostral region where a series of four postrostrals scales is present. Moreover, the tail is not proportional to the body. It appears as if the body of the gecko has become associated with a tail that does not belong to it.

Comparison. Palaearctic trihedral tuberculated geckos of genus *Tenuidactylus* and *Cyrtopodion* (see Khan & Tasnim, 1990 for complete list), differ from *Gymnodactylus walli* in having dorsal trihedral strongly keeled tubercles, both preanal and femoral pores in a continuous series; fewer (8–18) interorbital scales; 2–10 post femoral tubercles; caudal tubercles trihedral, six to a segment arising from the end of the segment. While Tibeto-Himalayan geckos of genus *Cyrtodactylus* (senso lato): *C. tibetinus*, *C. mintoni*, *C. dattanensis*, *C.* sp (Khan, in press), differ from *G. walli* due to plump round body and tail which are equal in length; unsegmented tail with reduced caudal tubercles and small subcaudals; 6–10 preanal pores; higher (106–205) midventral scale, and scales across midabdomen (30–54).

The high altitude gecko *Gymnodactylus stoliczkai* Steindachner, 1869, differs from *G. walli* in having 17–20 interorbital scales; 27–32 scales across midbelly, 120–149 along midventrum of body; no preanal or femoral pores; flat, strongly tapered tail, deeply sected laterally. In tail scalation *G. stoliczkai* and *G. walli* are similar to each other. There is distinct endolymphatic swelling on each side of head in *G. stoliczkai* and regenerated tail is much flattened.

Recently described sandstone geckos *Tenuidactylus indusoani* (Khan, 1988) and *T. rohtasfortai* (Khan & Tasnim, 1990), differ from *Gymnodactylus walli* in having much depressed and thin body, long tail with trihedral keeled tubercles, arising from the end of the segment, and a series of much broader subcaudals; body dorsum with flat, slightly keeled tubercles; fewer (13–16) interorbital scales; 21–33 scales across midabdomen; 103–135 mid-ventral scales; 6–7 preanal pores in *T. indusoani*, while a continuous series of 18–27 preanal and femoral pores in *T. rohtasfortai*.

Gymnodactylus walli is well differentiated by the following meristic characters:

Body moderately depressed; body dorsum with scattered oval keel-less tubercles, well differentiated from granular scales; subcaudals broader than long; small caudal tubercles arise from middle of caudal segment; 4–5 preanal pores:

..... *Gymnodactylus walli*

Body moderately depressed; body dorsum with large strongly trihedral keeled tubercles, arranged in longitudinal series; preanal as well as femoral pores; tail strongly segmented, caudal tubercles trihedral and keeled, subcaudals broad:

..... genus *Tenuidactylus*
and genus *Cryrtopodion.*

Body much depressed and thin; body dorsum with scattered flat, round feebly keeled tubercles; with preanal or both preanal and femoral pores in a continuous series; tail strongly segmented; caudal tubercles trihedral keeled, arising from the end of the caudal segment, subcaudal broad:

..... *Tenuidactylus indusoani*
and *T. rohtasfortai*

Body and tail round, plump; body dorsum with scattered, round to oval keel-less tubercles; only preanal pores; tail unsegmented, caudal tubercles small keelless flat structures, subcaudals not broader than long:

..... genus *Cyrtodactylus*

Body moderately depressed; body dorsum with scattered keel-less oval tubercles, slightly distinct from granular scales; no preanal or femoral pores; tail flat, laterally sected, caudal tubercles weak arising from the middle of the segment; subcaudals not broader than long:

..... *G. stoliczkai*
and *G. yarkandensis.*

Habitat. The specimens of *Gymnodactylus walli* from Ghariet, Chitral were collected from the walls of a roadside thatched house, near Ghariet village, in July, 1986, just after sunset. Chitral occupies the northwestern tip of Pakistan (Fig.2, inset). It stretches between 35°15' to 36°55'N, 71°21' to 73°55'E, over an area of 320 km of rugged mountains with an elevation ranging from 1500 m to 1850 m. (Adamson & Shaw, 1981). Maximum summer temperature 24°C, winter –50°C.

Two reports exist on the herpetofauna of Chitral, McMahon (1901) records *Spalerosophis diadema, Ptyas mucosus, Coluber rhodorachis, Echis carinatus, Naja naja, Xenochrophis piscator, Bungarus caeruleus, Amphiesma stolata, Lycodon striatus, Gongylophis conicus, Typhlops braminus, Varanus flavescens, Cyrotopodion scaber, Varanus bengalensis, Eublepharus macularius, Calotes versicolor*, While Wall (1911) records *Gymnodactylus stoliczkai* (= *G. walli*), *Agama tuberculata, A. himalayana, Varanus griseus, Liolopisma himalayana, Natrix tessellata, Coluber ravergieri, Agkistrodon himalayanus.*

DISCUSSION

Mertens (1969) reported SMF 63548 from Abbotabad, Hazara Division, NWFP, Pakistan, and identified it as *Gymnodactylus chitralensis*. Later Khan (1980) showed it to be *G. dattanensis*. Szczerbak and Golubev (1986), apparently unaware of Khan's work, followed Mertens' identification and along with SMF 63548 from Abbotabad they illustrated their account of *G. chitralensis* by MNHN 1916.63 and wrongly noted its type locality as "Upper Indus valley" (Golubev, pers. comm.), when in fact it is "Central Provinces, India" (Brygoo, pers. comm.). This specimen was identified by Chabanaud as *Gymnodactylus nebulosus* (1919, Bull. Mus. Nat. Hist. nat, Paris, 25, 452). Apparently Szczerbak & Golubev, (1986) were unaware of the presence of syntype of *G. chitralens* in the British Museum (Natural History), London, despite clear reference by Smith (1935) to its depository.

Ingoldby (1922) compared *Gymnodactylus walli* with *G. stoliczkai*. Smith (1935) disregarded the differences and pushed

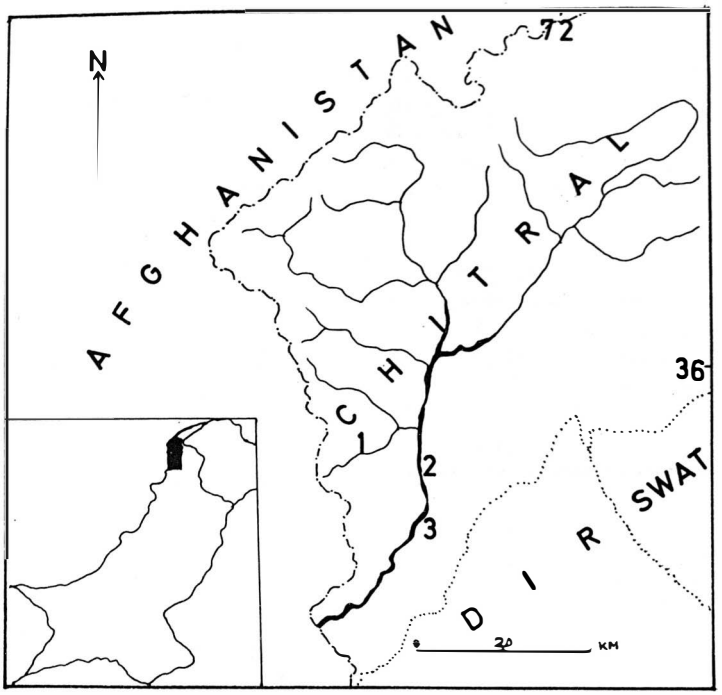

Fig. 2. Map of the part of Chitral, NWFP, Pakistan, showing collection localities of *Gymnodactylus walli* Ingoldby: 1=Bumburet (Bumhoet); 2=Ghariet; 3=Drosh Fort, (inset: Pakistan, shaded part showing position of Fig. 2).

G. walli in to the synonymy of *G. stoliczkai* to pave the way for his *G. chitralensis*. Ingoldby based his *G. walli* on a type series comprising of one adult male, two adult females, one half grown and one young specimen (1922, 1051). Logically he should have designated the adult male as type, but what we have as type is the youngest of the series, a juvenile about 1–2 week old (BMNH 1910.7.12.1.). There is no sign of preanal pores in this specimen neither it has been dissected for examination of its gonads, while in the description Ingoldby sexed it as male.

ACKNOWLEDGEMENTS

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APPENDIX: MUSEUM ACRONYMS

BMNH = British Museum (Natural History), London; MNHN = Museum National d'Histoire Naturelle, Paris; MSK = Herp Laboratory, Rabwah 35460, Pakistan; SMF = Senckenberg Museum, Frankfurt.

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FORAGING BEHAVIOUR OF THE BROWN TREE SNAKE, *BOIGA IRREGULARIS*

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(Accepted 22.4.91)

ABSTRACT

Boiga irregularis is a nocturnal, primarily arboreal, rear-fanged colubrid that is believed to have eliminated most of the native forest vertebrates on the island of Guam. On Guam it usually eats birds, rats, and lizards, including both day and night active species. To determine where the snakes forage, I tabulated 398 sightings of foraging snakes, recording their perch height, perch diameter, and perch plant species. These measures were compared to the places where searchers look for snakes, as well as the heights and perches where likely prey items are seen. Snakes were seen less often than would be expected based on search effort at heights from 2-5 m above ground. The modal height for foraging snakes was less than 0.5 m and they exhibited no preference for perch diameter. To determine how the snake locates its prey I watched 26 snakes for a total of 19.45 hours using a night-vision device. Both active search and ambush foraging modes were evident, with many snakes using both tactics within an evening. The postures adopted by immobile snakes suggest that they could detect the odor tracks of geckos. I also observed one medium-sized snake consume a sleeping adult columbid bird, which it found by active search.

INTRODUCTION

The brown tree snake, *Boiga irregularis*, has been associated with the loss of most native birds, bats, and lizards on the island of Guam (Savidge, 1987; Wiles, 1987; Fritts, 1988; Engbring & Fritts 1988). In addition, it has been held responsible for hundreds of power short-circuits (Fritts, Scott & Savidge, 1987), loss of agricultural and pet animals (Fritts & McCoid, 1991), and envenomation of human babies (Fritts, Scott & Smith, 1989; Fritts, McCoid & Haddock, 1990). Shortly after World War II the brown tree snake was accidentally introduced to Guam, an island lacking snakes as predators on vertebrates. By the 1980s the snake had reached localized population densities in excess of 50/ha (Fritts, 1988; Rodda, Fritts & Conry, 1992). Ongoing efforts to reverse or minimize the adverse impacts of the introduction have been hampered by lack of understanding of the behaviour of the snake.

Although the snake's predation on native vertebrates has attracted considerable attention, information on how the snake obtains these items has been limited to what may be inferred from stomach contents (Savidge, 1988; Greene, 1989; Shine, 1991). The snake had not been observed capturing prey in the wild. The gut samples indicate that the brown tree snake has a remarkably catholic diet, including all life stages (eggs, juveniles, adults) and all species of small terrestrial vertebrates. Its length (up to 3.0 m) also allows the consumption of smaller representatives of the larger vertebrate species. For example, on Guam the snake is well known for its attacks and feeding attempts on German shepherd puppies (Fritts, 1988). In general, smaller brown tree snakes consume mostly lizards while larger individuals eat primarily endotherms (Greene, 1989).

Both diurnal and nocturnal species are preyed upon; the snake is nocturnal (Fritts *et al.* 1987). These observations imply that both active and inactive prey items are taken. Likewise, the consumption of immobile bird and lizard eggs would seem to require an active foraging mode, whereas nocturnal rats and geckos might be most easily captured by ambushing. Although most snakes are thought to specialize in a particular foraging mode, the breadth of this snake's dietary proclivities suggested that it would exhibit both ambushing and active foraging modes.

The length of time that a foraging snake remains at a site ("giving up time") reflects its dependence on the ambushing tactic. Determining the "giving up time" of a foraging animal is also useful for testing the applicability of optimal foraging models (e.g. Schoener, 1971). In *Lachesis muta*, an ambushing pit viper, the "giving up time" is about two weeks (Greene & Santana, 1983). In contrast, actively foraging snakes such as *Nerodia* may move more or less continuously when foraging (Mushinsky & Hebrard, 1977). One objective of this study was to quantify the "giving-up time" of foraging brown tree snakes.

To design measures for control or eradication of the brown tree snake it is vital to know exactly where and how foraging takes place. Traps or capture programs directed at ground level may be futile if the snakes are foraging primarily in the forest canopy. Traps set on tree trunks might not work if foraging occurs primarily in foliage. In this paper I report on the distribution of snake sightings with reference to perch height, perch diameter, and perch plant species, both in absolute terms and in relation to the typical positions occupied by geckos, the snake's primary nocturnal lizard prey. In addition I report the moves and postures of foraging brown tree snakes, as observed with the aid of a night-vision device.

METHODS

As a consequence of recurrent typhoons and other habitat disturbances, most of the forests on Guam are short in stature (< 10 m). Therefore, brown tree snakes can be found by visually scanning trees at night. The snake sightings reported below were made at four forested sites on Guam: Orote Point plateau, Naval Communications Area Master Station overlooking Haputo Beach, along the road to Ritidian Point, and in the forest west of Northwest Field. In 398 observations of snakes made by myself or Renée Rondeau in Feb.-Oct. 1988, we estimated each snake's height above ground, perch diameter and the perch plant species. During the same censuses we also estimated the value of these parameters for all geckos seen (n = 890) and for the places that we were searching for snakes (n = 1611 spot samples). The spot samples consisted of our records of the plant species and perch heights that we were viewing, at the instant an unpredictable alarm sounded. A conscious effort was made to scan all vegetation and to scan it at a constant rate. While these data are only estimates, they give an indication of where most snakes were seen and whether these places also contained visible geckos. I quantified the positions of geckos not only because they are preyed upon by the snake, but also because the

observability of small geckos is less than that of brown tree snakes to a human searcher. All the geckos were relatively small; more than 95% were *Lepidodactylus lugubris* and *Hemidactylus frenatus*. A typical snout-vent length for a *Lepidodactylus* was 35 mm, that for *Hemidactylus* 45 mm, and that for *Boiga* 950 mm. Thus the decline in number of snake sightings at greater distances from the observer can be compared to that in more cryptic reptiles.

Using a night-vision device I watched 26 foraging snakes at various times between 14 February 1988 and 19 June 1989, for a total of 19.45 h. I used both monocular (Litton Industries M-845) and binocular (Litton Industries M-802A) devices, providing both magnification and photo-amplification. In some cases the naturally available light was augmented by light from adjacent street lamps; as the snakes had entered these dimly lit areas of their own volition, I assume that their behaviour was normal for these conditions. In the absence of moonlight, snakes were extremely difficult to detect with the devices in natural forest or on the ground. Lack of detectability was particularly a problem when the vine-like snakes stopped moving for long periods of time (e.g. hours). Under these circumstances I sometimes augmented the natural illumination with a stationary unfocused headlamp directed into the ground at an angle of at least 60° from the snake. This procedure did not greatly increase the ambient light level; the only light that reached the snake was that which was reflected off the dull soil or herbs, but it was enough additional light that the snake could be unequivocally detected with the photo-amplifying device (though not with the unaided eye).

During 1.33 h of observations I looked for evidence that the snakes' movements were disrupted by light. Snakes that had been directly illuminated by a moving light (i.e. not a fixed streetlamp) generally stopped moving while the light was on them and rapidly moved away from the light source after the illumination had passed. Snakes that were closely approached (approx. 2 m) with a light sometimes moved rapidly away. Therefore, only observations made without directly or closely illuminating the snake are included in the following results. With one exception (see below), the minimum viewing distance was approximately 7 m; a typical viewing distance was about 15 m.

Most of the observations (15 snakes; 14.08 h) were made on a chain link fence bordering the Naval Air Station-Agana. This site was chosen for the large number of snakes present and the relatively good visibility of snakes on the fence. The natural behaviour of these fence-climbing snakes is corroborated by a smaller number of observations (11 snakes; 4.04 h) made of snakes in the forest.

Foraging mode and predatory tactics may vary from site to site depending on prey availability. At the fence site, the most likely prey was geckos, both because the snakes were predominantly of a size that would eat geckos, and because geckos were extremely abundant on the fence. All types of prey were less abundant in the forest, and the average snake was larger in the forested areas (Rodda *et al.*, 1992). In the forest site, geckos may have been less important as a prey item than rats.

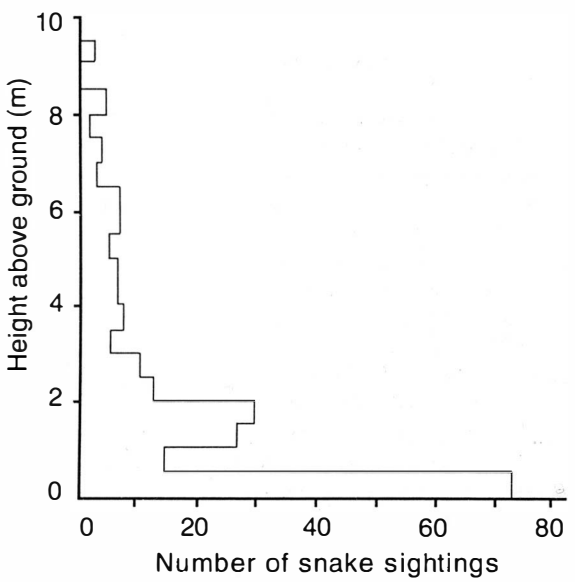

Fig. 1. Distribution of brown tree snake sightings by height above ground.

	Compared to places viewed			Compared to lizards seen		
Height(m)	Snakes	Viewed	Deviation	Snakes	Lizard	Deviation
0-1	41.4	37.2	4.1	13.6	15.9	-2.4
1-2	26.1	24.6	1.4	37.9	36.6	1.2
2-3	9.9	16.0	-6.1	14.3	19.3	-5.0
3-4	4.9	9.5	-4.6	8.6	12.0	-3.5
4-5	4.9	6.3	-1.4	7.1	9.1	-2.0
5-6	4.9	3.0	1.9	7.1	4.4	2.8
6-7	3.9	2.4	1.5	5.7	1.4	4.3
> 7	3.9	0.8	1.3	5.7	1.1	2.1
		$G_{adj} = 24.47$, $P = 0.0009$			$G_{adj} = 23.20$, $P = 0.0016$	

TABLE 1. Independence of height distributions between snake sightings and either visual searching effort or lizard sightings. For ease of interpretation the values in the body of the table are percent of sample; statistical results were based on the raw frequencies.

RESULTS

Height Above Ground

Although the brown tree snake readily ascends to great heights, we saw 77% of the snakes within 3 m of the ground (Fig. 1). Snakes that were foraging high in the trees were slightly more difficult to see than those closer to eye level, but the distribution of sightings (Fig. 1) is not entirely due to the greater difficulty of seeing snakes at greater distances. For example, consider two layers of the forest matched for distance from the observer's eyes, one 1-2 m above the eyes, the other an equal distance below: 41% of the total sample was seen in the lower level, whereas less than 5% was seen in the higher layer. This difference was not due to the snakes being easier to spot when they are viewed from above: the shiny yellow/white venter of a brown tree snake is more easily spotted than is the dull brown dorsum. However, the number of snakes seen at low heights may not be attributable to a preference of snakes for low heights: in the places searched (mostly roadsides) there is more vegetation at low heights. The distribution of plant heights along roadsides was not measured directly, but 78% of our searching time was devoted to plant surfaces below 3 m in height. Compared to the places viewed, more snakes were seen near the ground, and fewer snakes than expected were seen at heights from 2-5 m (Table 1). Compared to the heights where geckos were seen, the snakes were seen less often than expected within 1 m of the ground and at heights from 2-5 m. A higher proportion of the snakes than that of the lizards was seen at heights above 5 m (Table 1).

Perch Diameter

Brown tree snakes, even large ones, often crawl through the foliage, being supported by many small twigs. All sizes of snakes also readily crawl along stout limbs. This diversity of pathways is reflected in the absence of any correlation between perch diameter and snake length ($r = -0.03$, $P = 0.7$; Fig. 2). The perch diameters in Fig. 2 have been log transformed to obtain a normal distribution. Data from ground foraging snakes have been eliminated from this figure and the above correlation.

Plant Species

Table 2 shows significant independence between plant species viewed and plant species occupied by snakes. The distribution of plant species was marginally insignificant ($P = 0.08$) for the snake - lizard contrast. Compared to the places viewed, snakes were more likely to be seen on herbs and *Leucaena*, and less likely to be seen on "other trees". Most (93%) of the sightings in the herb category were on partially bare soil or close cropped grass, where visibility of snakes is exceptionally good. *Leucaena leucocephala* is a legume that folds its leaflets at night, which enhances snake visibility. In contrast to *Leucaena, Casuarina,* and *Scaevola,* the "other trees" had relatively dense foliage and therefore poor visibility for snake sightings.

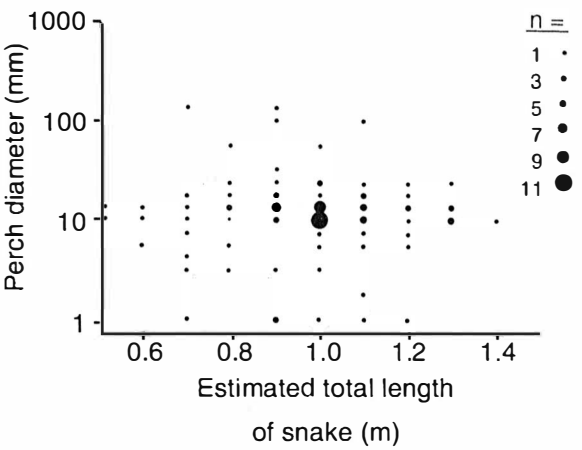

Fig. 2. Perch diameter in relation to snake size. Area of point represents frequency of sightings, as indicated.

	Compared to places viewed			Compared to lizards seen		
Plant type	Snakes	Viewed	Deviation	Snakes	Lizard	Deviation
HERBS	32.7	23.8	8.9	1.4	2.5	-1.1
VINES	3.9	4.0	-0.2	5.7	1.6	4.0
CASUARINA	2.0	2.2	-0.3	2.9	2.6	0.2
SCAEVOLA	6.3	4.5	1.8	9.3	5.6	3.6
LEUCAENA	47.8	43.3	4.5	70.0	75.4	-5.5
OTHER TREES	7.3	21.9	-14.6	10.7	11.9	-1.3
	$G_{adj} = 31.32$, $P = 0.000$			$G_{adj} = 9.72$, $P = 0.084$		

TABLE 2. Independence of plant species distributions between snake sightings and either visual searching effort or lizard sightings. Observations tabulated by major tree species and other plant habit types. Values are percentages of sample, analysed as in Table 1.

Direct Observations of Foraging Behaviour

Of the 26 snakes watched, four were discovered on the ground. Three of these were detected on nights with bright moonlight; these vanished in less than 15 seconds. The other was observed for 0.22 h without moonlight. This snake was the exception to the 7 m minimum viewing distance, as it could not be seen without closely approaching it. After a few minutes it appeared to detect the observer and fled rapidly. The 22 remaining snakes were all above ground when first detected. They were watched for periods ranging from 0.07 h to 2.62 h (mean = 0.87 h, S.D. = 0.68 h). Twelve snakes adopted a distinct ambushing posture at some time during the observations. This posture consisted of a motionless body more or less stretched out horizontally, with a distinct S curve in the neck region and the head held motionless very near (< 2 cm) a tree trunk or upright post. For ten of the twelve snakes seen in this posture, the snake either held this posture at the time it was first seen, or the snake maintained this posture until it was disturbed or observations were otherwise discontinued. Thus most of the recorded giving up times are minima. The twelve measured giving up times averaged 32 min (S.D. = 27 min), with the two uninterrupted ones being 10 min and 31 min in length. However, as shorter times would be more likely to be seen from start to finish, these complete intervals may not be representative. One snake was observed motionless in an ambush posture for 1 h 32 min before observations were discontinued.

Of the 22 snakes seen above ground, 16 crawled at some time during the observations. Crawling was usually accompanied by slow exploratory motions of the head, often with reversals of the prevailing movement direction. The net travel rate of the actively foraging snakes was low, and varied from 0 to 26 m/h (mean = 11.4 m/h, S.D. = 7.9). On only four occasions did I witness an undisturbed snake moving rapidly. In one case a snake climbed a fence very rapidly as a house cat approached. This was the only observed interaction with a non-human predator; the cat did not appear to detect the snake. On two occasions snakes bolted at the start of heavy rainfall. No obvious cause was associated with the fourth example of rapid movement.

One snake was observed to catch a prey item during observations. This snake was climbing the cross beam of a high voltage power line (inoperative at the time), when it seized a sleeping pigeon or dove by the head. The snake was about 1.2 m total length; thus the prey was relatively large for this snake. On the horizontal surfaces on which captive snakes are fed, brown tree snakes usually constrict prey that are large and struggling (personal observation; Chiszar, personal communication), but in this case the struggling bird fell off the beam and the snake appeared incapable of pulling it back up (the snake's tail was coiled around the beam as an anchor). After 22 min of the snake hanging off the beam with the bird in its mouth, the snake pulled the apparently dead bird back up to the beam and began swallowing it. Swallowing took 120 min, after which the grotesquely bulging snake began slow exploratory movements and I discontinued the observations.

DISCUSSION

Foraging Height

Although the brown tree snake has a morphology and locomotor skills associated with advanced arboreality (Chiszar, 1989), on Guam the snake appears to spend much of its time foraging on or near the ground (Fig. 1). This foraging behaviour probably reflects a relatively greater amount of foliage near the ground in the shrubby second growth areas characteristic of Guam. It may also reflect a dietary shift towards preying on skinks sleeping on the ground instead of the nocturnal arboreal geckos (which have been depleted in many areas of Guam: Rodda & Fritts, 1992). Savidge (1988) found that the most common item in brown tree snake stomachs from Guam was skinks.

The relatively large amount of foraging near the ground implies that measures to control the snake could be effective even if limited to heights easily reached by humans. Many snakes can be caught or trapped from ground level. However, substantial amounts of snake activity occurs at all levels in the forest (Fig. 1), and it is possible that individual snakes restrict their foraging to the canopy; thus total eradication may not be possible using exclusively ground level measures.

Perch Diameter

Unlike certain *Anolis* lizards (Scott, Wilson, Jones, & Andrews, 1976), brown tree snakes use all perch sizes (Fig. 2). For so large a snake the modal branch diameter was relatively thin (10-20 mm). The large number of slender branches in a forest and the diverse paths taken by the snakes suggest that brown tree snakes will not be concentrated along any particular pathway. Traps and other control measures may need to be placed in a wide variety of positions.

Plant Species

I interpret the distribution of plant species to reflect primarily the visibility of snakes (Table 2). *Leucaena*, for example, may have a greater proportion of snakes, or the snakes that are on *Leucaena* may simply be more visible. Without additional information indicating a concentration in certain plant species, it may be best to distribute traps and other control measures on a wide variety of plant species.

Foraging Mode

Brown tree snakes exhibit both ambush and active foraging modes on a regular basis. Most snakes appeared to use both modes on a single night. Brown tree snakes may maintain an ambushing posture for several hours, but most also moved during a night. The active foraging mode facilitates the applicability of control measures, whereas ambushing snakes would have relatively few opportunities to encounter a trap or other control device. It may be advisable to bait traps with prey stimuli that are appropriate to active foraging modes (e.g. bird odors), as opposed to stimuli appropriate to ambush mode (e.g. geckos). However, Rodda, Rondeau, Fritts & Maughan (1992) found that gecko baited traps were more successful than similar traps baited with bird odors. Perhaps the bird odors used were a weaker prey stimulus than a live gecko. Much more information is needed on the attraction stimuli needed to maximally entice foraging snakes to enter traps. For example, the foraging snakes appeared to choose the sites at which to adopt an ambushing posture. Do they identify these sites based on the odor trails left by the passage of geckos or other food items? Fritts (pers. comm.) found that brown tree snakes could detect the prior passage of another brown tree snake, presumably by olfactory means. Thus it seems plausible that brown tree snakes could also detect the prior passage of prey items, as Chiszar, Melcer, Lee, Radcliffe & Duvall (1990) has shown for *Crotalus viridis*.

In summary, the brown tree snake appears to be a very adaptable forager, capable of taking a wide variety of prey from a wide variety of places using a variety of foraging tactics. The snake's success as an invader on Guam may in part be due to this adaptability. To the wildlife manager, this variability offers diverse opportunities for attracting and contacting this pest species. The drawback to the brown tree snake's adaptability is that efforts to exclude the snake will necessitate blocking a multitude of pathways.

ACKNOWLEDGEMENTS

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AERIAL AND AQUATIC RESPIRATION IN THE BLACK-RAYED SOFTSHELL TURTLE *AMYDA CARTILAGINEA*

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ABSTRACT

Black-rayed softshell turtles (*Amyda cartilaginea*) from Malaysia were shown to be capable of extracting oxygen from water by a combination of cutaneous and buccopharyngeal respiration. Given access to air as well as water they consumed a mean 81 ml O_2 $g^{-1}h^{-1}$; when submerged and respiring aquatically the uptake fell to a mean value of 21 ml O_2 $g^{-1}h^{-1}$ (ratio 3.86:1). Behavioural data show that the turtles cannot survive indefinitely by aquatic respiration alone as they incur an oxygen debt, even when inactive. Scope for activity is substantially reduced, even when air becomes available, until the oxygen debt is repaid. Buccopharyngeal respiration is a normal feature of behaviour, and is not used solely during prolonged submergence. The turtles pump some 40-80 ml water min^{-1} through the pharynx at 30°C. Turtles display dilated cutaneous blood vessels when they are submerged for long periods.

INTRODUCTION

Reptiles are in general air breathers, which rely wholly upon pulmonary gas exchange. However, over the past century a number of aquatic chelonians have been shown to gain some of their oxygen directly from the surrounding water. Gadow (1901) described the vascularized cloacal sacs of emydid turtles through which water is circulated, while Cahn (1937) demonstrated that water was moved in and out of the pharynx by emydid chelonians of the genus *Chrysemys*. Most interest in chelonians has centred upon the soft-shelled turtles (Family Trionychidae). Softshells are specialized, highly aquatic turtles, rarely seen out of water. They have flattened, skin-covered shells, long necks and distinctive snorkel-like snouts which enable them to breathe inconspicuously at the water surface. Gage & Gage (1886) described filamentous pharyngeal viliform processes in *Trionyx* (= *Amyda*) *spinifer* LeSeuer and *Trionyx muticus* LeSueur, and also showed that these animals could take up oxygen whilst under water. They believed that the viliform processes functioned as gills, and were responsible for most aquatic oxygen uptake. Several authors have noted that the thin, well-vascularized skin covering the shells of softshells is likely to function as a respiratory surface; as Pritchard (1979) remarks 'the soft, delicate skin would seem to be somewhat of a liability otherwise'. Three decades ago two workers (Dunson, 1960; Girgis 1961) demonstrated that some softshell species (*Trionyx spinifer* and *Trionyx triungis* Forskal) could definitely extract oxygen from water (earlier workers other than Gage and Gage had assumed a respiratory function for skin and buccopharynx, but presented no direct evidence in support of the hypothesis). Girgis (1961) claimed that aquatic uptake of oxygen was sufficient to support the metabolism of an inactive, submerged Nile turtle.

The study reported here was carried out on a softshell species which has previously attracted little study, none of it physiological. The black-rayed softshell turtle *Amyda cartilaginea* is a large species (< 70 cm carapace length) widely distributed in S.E. Asia (Pritchard, 1979). It lives in muddy rivers as well as clear hill streams and is usually described as carnivorous, though a dead specimen dissected for the present study had a large intestine packed with palm kernels, suggesting a more omnivorous lifestyle. Like many other Asian softshells, the species is exploited commercially for its flesh, being caught in traps or by rod and line. Black-rayed softshells were studied to determine whether they were able to take up oxygen from water, to assess the importance of such uptake, and to investigate the ventilatory behaviour, both pulmonary and buccopharyngeal, before, during and after periods of submergence.

MATERIALS AND METHODS

Collection and Maintenance

Four animals were purchased from a food market in Penang, Malaysia; they had been collected from streams on the west coast of peninsular Malaysia. One had been badly bitten by the others and died shortly afterwards; it was used in anatomical investigations. The rest were held in fresh water at 30±2°C and fed three times per week on chicken liver and trash fish. They were returned to the wild at the conclusion of the study.

Apparatus

Breathing patterns and oxygen uptake in air and water were studied with the aid of the perspex apparatus shown in Fig. 1 which was held in a temperature bath. All experiments were carried out at 30°C. The turtles could each be exposed to four experimental situations (modes A-D). In mode A the animal was supplied with flowing, aerated water (< 2 l min^{-1}) and had access to an air space (volume 3096 ml). The animal's breathing in air and pharyngeal pulses under water could be observed. In mode B an oxygen electrode connected via a pH meter (both manufactured by Strathkelven Instruments) to a chart recorder was fitted to lid 1 of the apparatus. This arrangement allowed the measurement of oxygen tension within the air space. To make an oxygen uptake measurement, a turtle was settled within the apparatus for 2 hr with water flowing through the apparatus (as in mode A). The flow was then cut off by closing taps 1 and 2. Magnetic stirring of the water continued so that the aquatic and aerial phases were in equilibrium and the animal was free to take up oxygen from both phases. In mode C, lid 2 was applied so that the animal could only take up oxygen from water, but water still flowed through the apparatus. Finally, in mode D, lid 2 was fitted with the oxygen electrode and taps 1 and 2 were

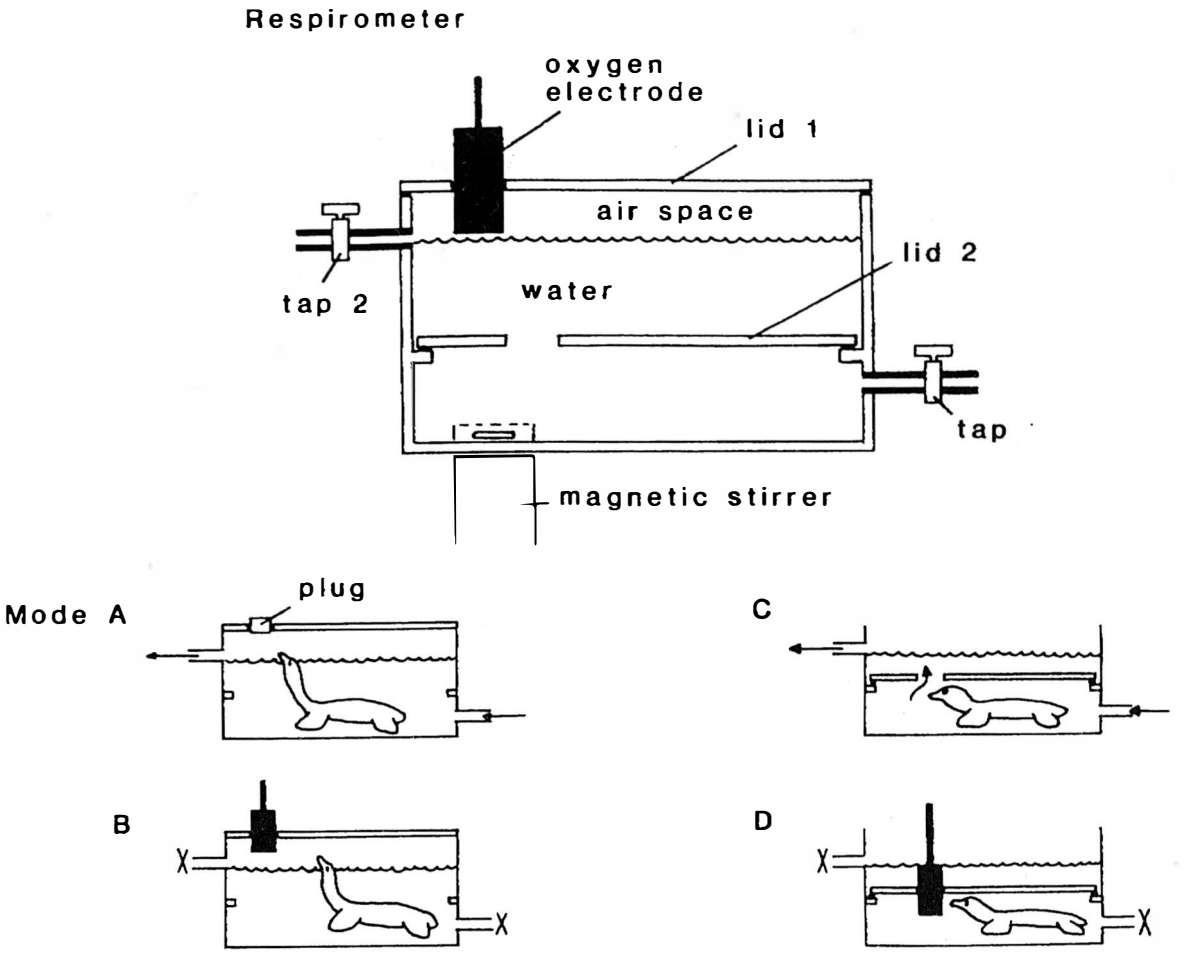

Fig. 1. Apparatus used in behavioural and respirometric studies. The four modes of use of the apparatus are also illustrated.

closed so that aquatic oxygen uptake could be measured. For modes C and D, lid 2 was gently put in position without disturbing the animal under investigation.

Experimental Protocol

Behavioural responses. The three turtles, in turn, were each studied before (apparatus mode A), during (mode C) and after (mode A) a period of sustained immersion. The following features of behaviour were monitored throughout; physical activity (measured as the number of movement cycles exhibited by the left forelimb per min), the number of breaths taken at the surface, the number of times the animal surfaced (i.e. projected part of the head above the water surface), and the number of pharyngeal pulses per min. Turtle 1 was held immersed for 90 min, turtle 2 for 120 min and turtle 3 for 50 min. At intervals, in all experiments, the appearance of the unpigmented plastron was inspected with the aid of a mirror to determine the degree of vasodilation.

Oxygen uptake measurements. Each turtle, in turn, was subjected to the following procedure. First the animal was allowed to settle in the apparatus for 2 hr (mode A). Next the oxygen uptake in both air and water was measured over a period of 4 hr (mode B). The animal was then returned to the mode A configuration for 2 hr. Finally, aquatic oxygen uptake was measured over a period of 20-30 min (mode D). Due allowance was made for the volume of the animal (measured by displacement) in all calculations. No attempt was made to measure aerial oxygen uptake in softshells held out of water as this risks skin damage and the animals usually exhibit prolonged activity.

Anatomical Observations

Live animals. To determine the water flow characteristics associated with pharyngeal pulsations, a vital dye (methylene blue) was introduced into the water around the snout of a softshell resting on the bottom of its holding tank.

To estimate the tidal volume of water exchanged with each pharyngeal pulsation, a second softshell was filmed when respiring underwater (with a Panasonic F10 videocamera fitted with a high speed shutter) from in front and from the side against a 1 cm grid background. To make measurements, fine fibretip drawings were made from frozen videofields by placing acetate sheets over a video monitor screen.

Dissection. A dissection and latex injection of the anterior arterial system was carried out to determine whether *Amyda cartilaginea* possesses the rich pharyngeal vascularization described for *Trionyx spinifera* by Gage & Gage (1886) and for *Trionyx triunguis* by Girgis (1964).

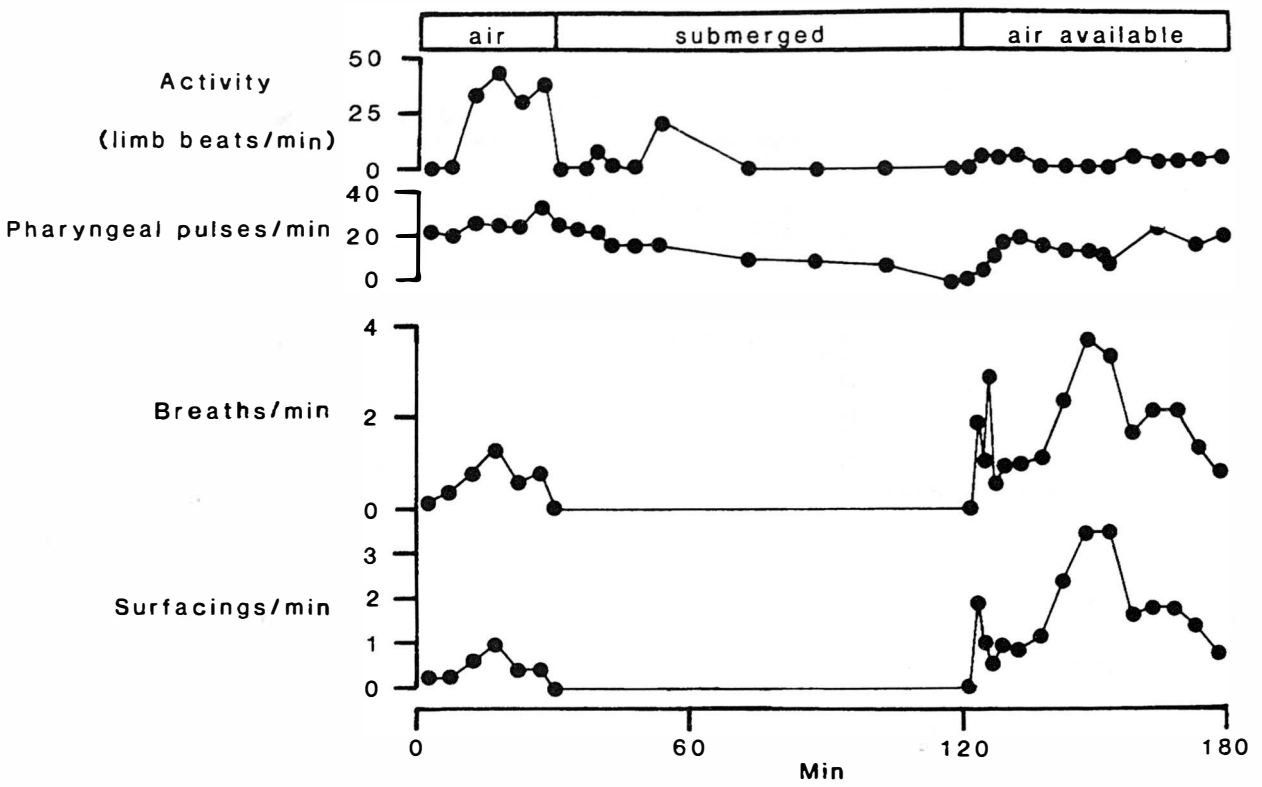

Fig. 2. Patterns of behaviour of turtle 1 before, during and after prolonged submergence.

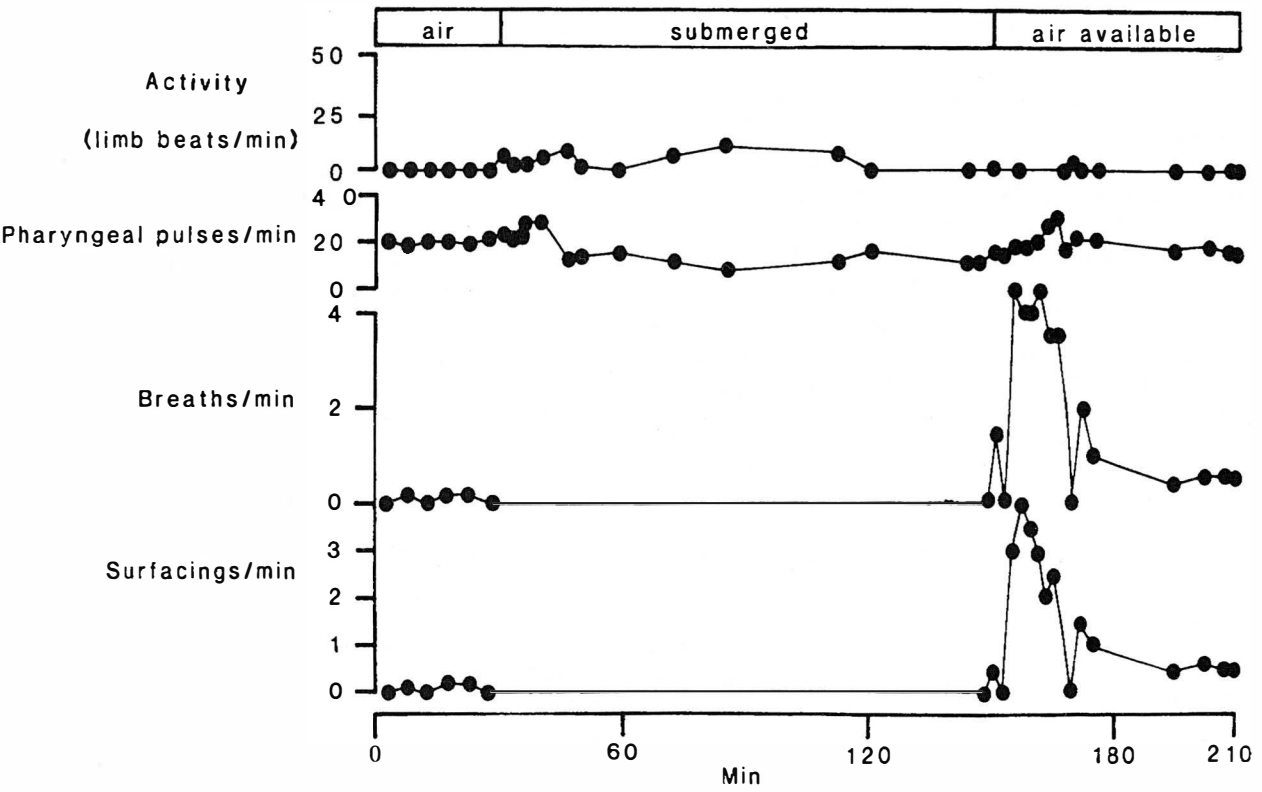

Fig. 3. Patterns of behaviour of turtle 2 before, during and after prolonged submergence.

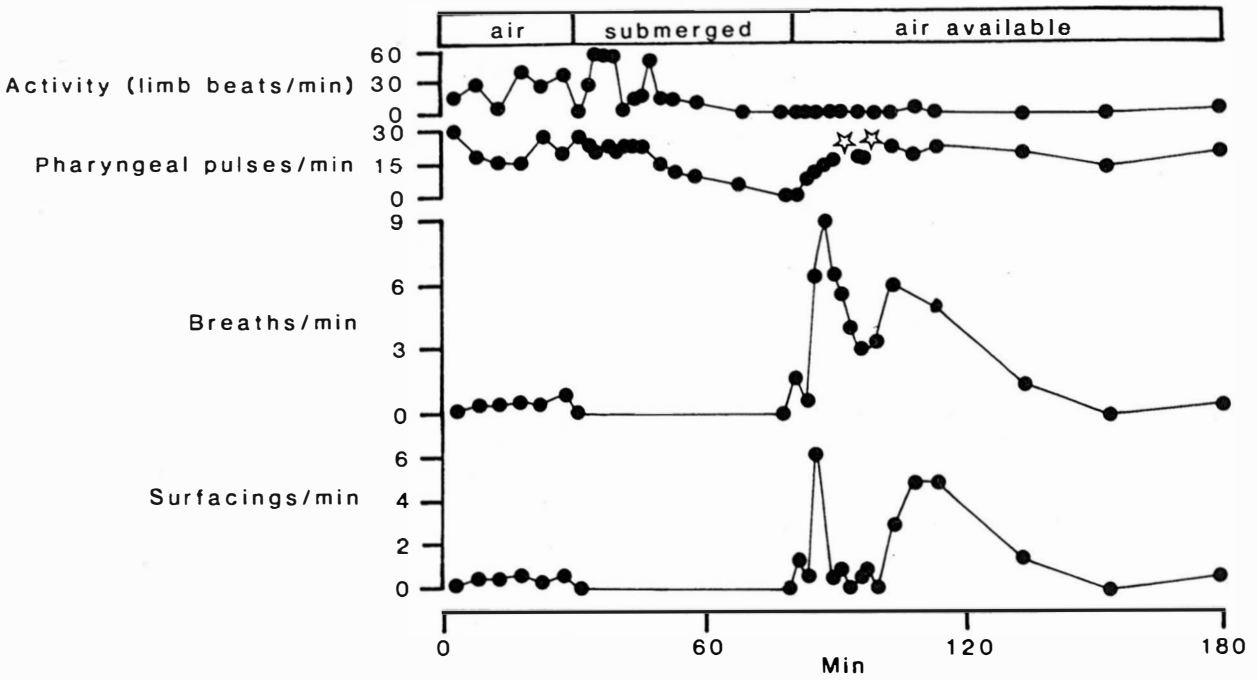

Fig. 4. Patterns of behaviour of turtle 3 before, during and after prolonged submergence. The stars indicate periods of time when the animal maintained its head out of water so that pharyngeal respiration was impossible.

RESULTS

Behavioural Responses to Immersion

Figs 2-4 show the responses of the three turtles. Animal 1 (Fig. 2) showed a burst of swimming activity in the 20 min period prior to submergence. During this activity there was a rise in pharyngeal pulsation rate (from about 20 to 34 pulsations min^{-1}), while the breathing rate rose from 0.2 breaths min^{-1} to a peak of 1.4 breaths min^{-1}. During submergence physical activity fell to zero within 40 min. The pharyngeal pulsation rate fell steadily, reaching zero after 87 min. By this stage the normally greyish-white plastron was noticeably pink in colour and dilated surface blood vessels could be discerned. On gaining access to air, the turtle showed very little swimming activity, but the animal immediately surfaced several times by extending its neck and head to the surface (usually taking more than one breath per surfacing episode). After taking a number of deep breaths in the first 5 min after gaining access to air, the breathing rate of the animal dropped back to 0.5 breaths min^{-1}. Over the next 20 min the breathing and surfacing rates rose, breathing reaching a maximum of 3.8 breaths min^{-1} - far higher than during the the burst of activity before submergence. Pharyngeal pulsations restarted as soon as breathing recommenced, and within 10 min had returned to 20 pulsations min^{-1}. The breathing rate fell over a further 40 min to less than 1 breath min^{-1}, and the animal again began to breathe only once per surfacing. This sequence showed that (a) pharyngeal pulsations were more frequent during activity, indicating that aquatic respiration was a normal feature of respiration, and not limited to periods of prolonged submergence, and (b) that the greatly enhanced rate of breathing after the 90 min submergence period represented repayment of an oxygen debt (since the turtle showed negligible physical activity when the rapid breathing was taking place). However, because the turtle had been active before submergence, it was not possible to be certain that the oxygen debt had been incurred during submergence.

Animal 2 (see Fig. 3) was inactive for a long time before submergence, showed little swimming during the 120 min of immersion (during which period the pharyngeal pulsation rate fell by half), but this animal still showed an enormous increase in breathing rate (to a maximum of 4.5 breaths min^{-1}) after gaining access to air. This response confirms that the the increased ventilation after submergence reflects an oxygen debt acquired during that submergence. Turtle 3 (Fig. 4) was active before submergence and for the first 20 min of immersion. Activity and pharyngeal pulsation rate then fell gradually to zero. On gaining access to air the animal exhibited a rapid increase in breathing rate (to 9 breaths min^{-1}) in the first 10 min, followed by a fall, a rise and then a slow decline in breathing rate so that by 70 min after regaining access to air, the breathing rate had returned to resting levels and the animal began to swim again, albeit at a very low level of limb beat. As with the other turtles the pharyngeal pulsation rate rose to 20 pulsations min^{-1} within about 20 min of gaining access to air, but there was no sign of an overshoot in pharyngeal respiration, indicating that an oxygen debt is paid off by aerial respiration alone. Turtle 3 exhibited a pink plastron at the end of the period of submergence; the colour had disappeared by the time breathing rates had returned to normal.

Oxygen Uptake

Oxygen uptake was recorded in two circumstances; when oxygen in both air and water was available, and when dissolved oxygen alone was accessible. From Table 1 it may be seen that oxygen uptake is much higher when air is available, by a factor of 3-6 (mean 3.9:1). The mean aquatic oxygen uptake rate (21 $\mu l\ O_2\ g^{-1}h^{-1}$) recorded was about three times the level reported by Girgis (1961) for specimens of *Trionyx triunguis*. However, the latter were bigger animals (605-1520 g) and were investigated at a lower temperature (23°C), so this difference is probably not significant.

Animal No.	Body wt (g)	Oxygen uptake in air + water (ml O_2 $g^{-1}h^{-1}$)	Oxygen uptake in water alone (ml O_2 $g^{-1}h^{-1}$)
1.	371	68	23
2.	310	102	16
3.	326	72	25
	mean	81	21
	ratio	3.86	1

TABLE 1. Oxygen uptake in *Amyda cartilaginea*

ANATOMICAL OBSERVATION

Dye tests showed that there was two-way movement of water through the external nares, that some inhalation was through the mouth, but that exhalation (more forceful) was mainly through the nose. Videotape records showed that the pharyngeal region of the animal studied (turtle 3, 326 g body weight) changed in volume by about 2-4 ml between maximum inhalation and maximum exhalation. This suggests that, at rest, the animal would have been moving about 40-80 ml of water through the pharynx per minute. Roughly speaking this would result in 12-24 ml O_2 passing over the pharyngeal epithelium per hour.

Given an aquatic oxygen uptake rate of about 8 ml O_2 h^{-1} for this turtle (see Table 1), it is conceivable that aquatic respiration could be sustained by buccopharyngeal uptake alone (though this would imply a rather high extraction efficiency and steep gradients of oxygen tension between blood and water).

Dissection showed that the vascular arrangements in the pharyngeal region of *Amyda cartilaginea* are very similar to those described for the Nile softshell by Girgis (1964). Paired pharyngeal arteries supply a rich arterial vascularization in the floor of the pharynx (a 'rete mirabile'); the pharyngeal arteries arise from the carotids.

DISCUSSION

Amyda cartilaginea like other softshelled turtles, is clearly capable of extracting oxygen from water. It may also be seen from the breathing pattern data that the animals would not be able to survive indefinitely by aquatic respiration alone, even when inactive, since a considerable oxygen debt (presumably reflecting accumulation of by-products of anaerobiosis) is incurred during prolonged submergence. Since resting animals with access to air consume oxygen at nearly four times the rate of animals able to take oxygen only from water, this finding is not unexpected, but conflicts with the conclusion of Girgis (1961) that Nile softshells could sustain an inactive metabolic rate by aquatic respiration alone. Certainly the evidence presented here strongly suggests that *Amyda cartilaginea* cannot remain submerged for periods of more than a few hours at most, and the resulting oxygen debt will restrict scope for activity for a considerable period thereafter.

Previous investigators have suggested that aquatic respiration in softshells is an inefficient process, only of survival value during prolonged submergence (e.g. Girgis, 1961). There has also been debate about the relative contribution of cutaneous and buccopharyngeal oxygen uptake (Dunson, 1960; Girgis, 1961).

As far as overall efficiency is concerned, the aquatic respiration rate of *Amyda cartilaginea* corresponds to about a quarter to one third of the aerial respiration rate (the precise value is difficult to assess because in the experiments reported here 'aerial' oxygen uptake probably included some aquatic uptake of oxygen). This proportion is appreciably greater than that recorded by Root (1949) for hard shelled musk turtles, in which the aquatic oxygen uptake rate was only about 12% of the aerial value. This finding reinforces the idea that softshelled turtles are especially adapted for a life in which surfacing is reduced to a minimum.

The behavioural data collected in the study reported here clearly indicate that buccopharyngeal respiration is a normal feature of this species whenever the head is under water. Because the pharyngeal pulsation rate rises with increasing activity, it seems probable that uptake of oxygen by the pharynx is an auxiliary respiratory function at all times; its existence will delay a return to the surface for air, whether this be at intervals of a minute or so during violent activity, or at hourly intervals when the animal is quiescent. Girgis (1961) argued that pharyngeal uptake could not be important except during prolonged submergence, because the blood supply to the pharyngeal rete was from the carotid arteries, so would be fully oxygenated and therefore would not allow uptake of oxygen from water. However, Agassiz (1857) long ago showed that softshell turtles have unusually small lungs as far as chelonians are concerned. In consequence, the oxygen tension in alveolar air is likely to decline rapidly after the turtle takes a breath (particularly when the animal is active), so that arterial pO_2 will also fall, allowing oxygen uptake across the villi of the pharyngeal lining. As far as hard shelled chelonians are concerned, Vos (1936), McCutcheon (1943) and Root (1949) all considered that flow of water in and out of the pharynx was primarily carried out for olfactory purposes. The findings reported here for *Amyda cartilaginea* definitely indicate a respiratory rôle; not only is the rate of water flow through the buccopharyngeal region much greater than would be needed for olfaction, but the rate of pulsation is linked to activity.

While Dunson (1960) argued that buccopharyngeal oxygen uptake made the major contribution to aquatic respiration (in *Trionyx spinifer*), and that cutaneous uptake was less important, Girgis (1961) conducted experiments which appeared to show that 70% of the aquatic oxygen uptake in *Trionyx triunguis* took place across the skin of the shell, limbs and body, while only

30% of uptake was by buccopharyngeal respiration. Unfortunately the design of Girgis' experiments was flawed, so it is impossible to be confident about his results. Girgis held turtles in water-filled, sealed tanks that were divided into two compartments, one containing the head and neck, the other the rest of the turtle. Oxygen concentrations (not tensions) in both compartments were measured. The underlying rationale behind the experiment was that oxygen uptake in each compartment was independent. However, blood flow around the body provides a route of gaseous exchange between the two compartments. Given that the pharynx and body skin are both permeable to oxygen, it may be predicted that the oxygen tension in the two compartments (and the body fluids) will tend to equilibrate, so that the apparent oxygen uptake from each compartment will simply reflect the size of the compartments - as is the case if Girgis' data and experimental technique are inspected carefully! In the present study no attempt was made to differentiate between the uptake across pharynx and integument, not least because it is difficult to see how this could be done in an unstressed animal. The technique pioneered by Root (1949) on the hardshelled musk turtle (*Sternotherus odoratus* (Latreille)) involved sealing off the body surface so that only buccopharyngeal respiration could take place. Girgis also employed this method on the Nile softshell, but in both cases the assumption that the animal will extract oxygen across the buccopharyngeal wall at the same rate as it would if other possible respiratory surfaces were functioning is rather dubious. However, in the case of *Amyda cartilaginea*, some indirect assessments may be made. Firstly, during prolonged submergence, the pharyngeal pulsation rate tends to decline, sometimes to negligible levels, while the plastron surface shows evidence of increased cutaneous blood flow. This suggests that the balance between cutaneous and buccopharyngeal uptake is a dynamic one, with uptake across the skin becoming more important with duration of submergence. Secondly, the rate of water flow through the pharynx, combined with the measured oxygen uptake when submerged, suggests that the black-rayed softshell would have to extract about one-third to two-thirds of the available oxygen from the flow of water if it was to rely on buccopharyngeal exchange alone. Such efficiency of extraction seems most unlikely, despite the two-way flow of water, so it is probable that cutaneous uptake was at least as important as buccopharyngeal gas exchange in the experiments reported here. On the other hand, it needs to be remembered that softshells, *Amyda cartilaginea* included, are burrowing forms in their natural habitat. When much of the body surface is covered with sand or mud, it will be impossible for significant amounts of oxygen to be taken up across the skin. Indeed, since the interstitial pO_2 of sediments is usually low due to a combination of microbial respiration and poor water circulation, it may be necessary for the turtle to constrict peripheral blood vessels to avoid loss of oxygen to the sediment. In these circumstances the buccopharyngeal route is likely to become dominant.

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ON THE LIFE HISTORY OF THE CAECILIAN GENUS *URAEOTYPHLUS* (AMPHIBIA: GYMNOPHIONA)

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ABSTRACT

Previous workers have suggested that uraeotyphlid caecilians are probably oviparous with direct development. Contrary to these suggestions *Uraeotyphlus oxyurus* has a larval stage with typically larval morphological features including a lateral line system, 'spiracles', and labial folds. Two larvae and one metamorphic specimen of *U. oxyurus* are described and aspects of their morphologies compared to that of adult *Uraeotyphlus*, the larvae of other caecilians and to that of aquatic adults of the Typhlonectidae. Gut contents indicate that the larva of this species is not a highly abbreviated non-feeding life history stage.

INTRODUCTION

The monogeneric caecilian family Uraeotyphlidae comprises four nominate species from the state of Kerala, South India that share many primitive morphological attributes with caecilians of the 'primitive' families Rhinatrematidae and Ichthyophiidae, but which are thought to be closer cladistically to the more derived 'higher' families Scolecomorphidae, Typhlonectidae and Caeciliaidae (Nussbaum 1979; Duellman & Trueb, 1986). As far as is known, rhinatrematids and ichthyophiids are oviparous and have a free-living larval stage whereas the majority of species of the higher families are either oviparous with direct development or are viviparous (Nussbaum, 1977; Wake, 1977).

Several opinions have been expressed concerning the life history of *Uraeotyphlus*. Ramaswami (1941) described the cranial anatomy of *U. narayani* and noted that the smallest specimen examined by him (a 90 mm juvenile) was essentially adult in its morphology. He consequently included among a list of characters distinguishing *Uraeotyphlus* and *Ichthyphis* the "highly abbreviated embryonic and larval periods and the appearance of adult characters very early in larval life if not in the embryos of *Uraeotyphlus*" (Ramaswami, 1941 p. 198). Wake (1977) listed *U. oxyurus* among the oviparous caecilian taxa as evidenced by unreported clutch data, and presumably based on the observed correlation between oviparity, large ova mass and large clutch size in caecilians for which more direct information on life histories is available. Nussbaum (1979) cited Ramaswami's (1941) observations as strong support for the inference that *Uraeotyphlus* has direct development with no free-living larval stage. Additionally, he noted that dissections had revealed large yolky eggs, typical of oviparous caecilians, and no foetuses. Neither of the latter workers cited Parker & Dunn's (1964) inclusion of *U. oxyurus* in a list of caecilians with free living larvae.

Nussbaum (1979) included the absence of a larval stage as a derived state in a cladistic analysis of the phylogenetic position of *Uraeotyphlus* relative to twelve other caecilian genera. Contrary the text, *Uraeotyphlus* was scored as having the primitive state of this character in his data matrix. Duellman & Trueb (1986) recapitulated the same contradiction by describing *Uraeotyphlus* as presumably having direct development, but then scoring the Uraeotyphlidae as having larvae for the purposes of cladistic analysis of familial relationships within the Gymnophiona. Lescure *et al.* (1986) scored *Uraeotyphlus* as having direct development for the purposes of developing a generic level phylogenetic hypothesis for caecilians. There is thus considerable confusion in the literature concerning the life history of *Uraeotyphlus* which requires clarification.

In 1882, the British Museum (Natural History) [BMNH] purchased three specimens of *Uraeotyphlus oxyurus* from Col. Beddome that are listed as larval specimens in the Museum's register. One of these specimens was exchanged with G. K. Noble and the American Museum of Natural History [AMNH] in 1925. The two BMNH specimens were almost certainly seen by Parker, and presumably formed the basis for inclusion of this species in Parker & Dunn's (1964) list. I have recently examined all three of the specimens collected by Col. Beddome over a century ago.

MATERIALS AND METHODS

Specimens were examined with the assistance of a binocular dissection microscope. All measurements were made to the nearest 0.1 mm with dial callipers except total lengths which were measured to the nearest 1 mm by stretching the specimens along a ruler. Short incisions were made in the posterior gut and urodeal part of the cloaca to allow examination of ingested material. Figures were prepared from camera lucida drawings.

RESULTS

The smallest of the three specimens, BMNH 82.12.12.11 is unquestionably larval. It has a total length (TL) of 87 mm, and differs most obviously from metamorphosed specimens in the possession of labial folds that are associated with aquatic suction feeding (O'Reilly, 1988), external nares that are lateral and subtriangular rather than dorsal and subcircular, and a pair of small but well defined spiracles, one on each side (Fig. 1a).

The labial folds are developed along the lateral margins of both the lower and upper jaws. With the mouth closed or only slightly opened, the upper fold extends over, and lateral to, the lower fold. Close to the angle of the jaws, upper and lower folds interdigitate and are probably not normally separated in life. Rostrally the upper folds gradually diminish in size and eventually completely disappear, leaving a small anteromedial oral aperture that is unguarded by labial folds (Fig. 2a). The form of the labial folds would clearly concentrate the full suction effect of buccal expansion to the region of the anteromedial oral aperture during feeding.

The spiracle of larval caecilians is associated with the distal tips of the third and fourth ceratobranchials and is therefore not

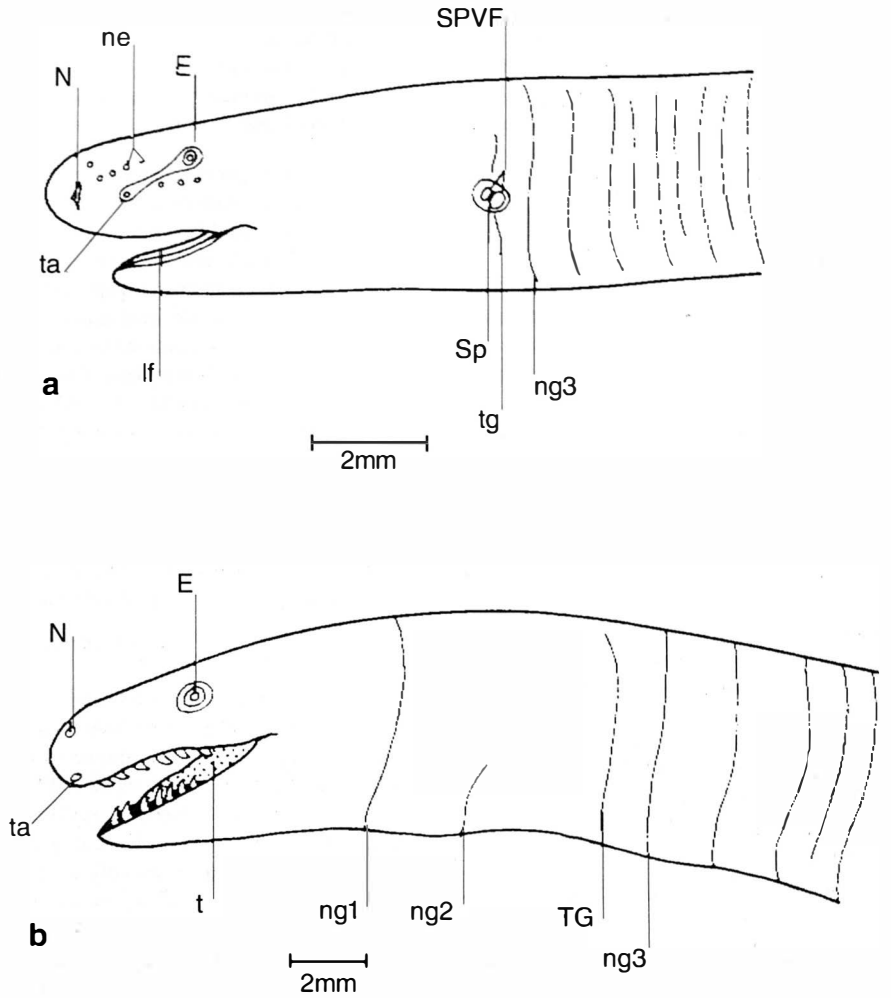

Fig. 1. Comparative lateral views of the head and anterior trunk of (a) larval (AMNH 23659) and (b) adult (BMNH 82.12.12.10) specimens of *Uraeotyphlus oxyurus*. Abbreviations: E, eye; lf, labial fold; N, naris; ne, neuromasts; ng1 - ng3, 1st - 3rd nucal groove; Sp, spiracle; SPVF, spiracular 'valve' flaps; t, tongue; ta, tentacular aperture; tg and TG, transverse groove.

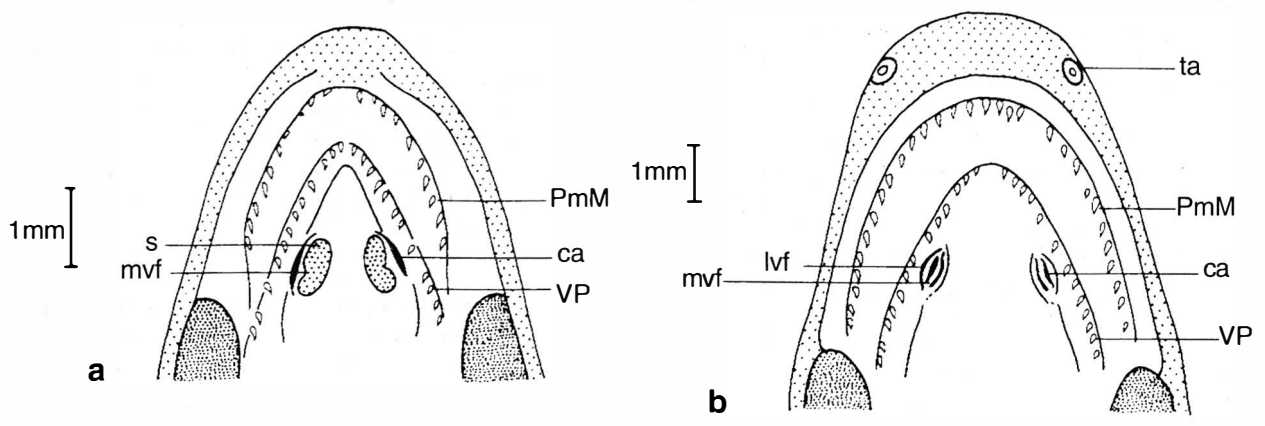

Fig. 2. Comparative palatal views of the buccal cavity of (a) larval (AMNH 23659) and (b) adult (BMNH 82.12.12.10) specimens of *Uraeotyphlus oxyurus*. Abbreviations: ca, choanal aperture; lvf, lateral choanal valve flap; mvf, medial choanal valve flap; PmM, premaxillary-maxillary tooth series; s, sac-like medial expansion of medial choanal valve flap; ta, tentacular aperture; VP, vomeropalatine tooth series.

homologous with the spiracle of elasmobranchs. Each spiracular aperture is guarded by a pair of well developed fleshy flaps that are continuous with the surrounding skin. These fleshy, valve-like flaps make the spiracular aperture narrow and elongate (Fig. 1a) and presumably aid in closure of the spiracle. There is no indication of gills, although the spiracular area extends as a depression a little anterior to the aperture and this anterior region was presumably equipped with the external gills, that may be assumed to characterise an earlier stage in the ontogeny of this species.

The tentacular aperture is far anterior to the eye but posterior to the level of the naris. The eye and tentacular aperture are connected by a faint eye-tentacle stripe that indicates the position of the tentacle organ under the dermis. In adult *Uraeotyphlus* the tentacular aperture is further anterior, directly below, or below and slightly anterior to the dorsal naris, and the tentacle organ is covered by the maxillopalatine with no indication of an eye-tentacle stripe (Fig. 1b). Also unlike the adult condition there are no scales associated with the relatively poorly marked annuli and the skin is distinctly thinner and less glandular. Although a full adult complement of annuli are present in the larva, the nuchal collars are not clearly differentiated (Fig. 1). There are further differences between this larva and adults in characters of the buccal cavity (see below).

The larva also differs from those of ichthyophiids and rhinatrematids described by Taylor (1968, 1970) and Hetherington & Wake (1979) and from the larvae of the caeciliaid *Sylvacaecilia grandisonae* described by Largen *et al.*, (1972). There appears to be no indication of a lateral line system and the tail bears no fin and lacks any substantial lateral compression.

A second specimen AMNH A23659 is slightly larger (TL 85 mm) than the former but is also distinctly larval. Despite its slightly larger size, this specimen appears less developed than the former in having the tentacular aperture distinctly closer to the eye and therefore further from the adult position. There are also a few poorly indicated neuromast organs on the head belonging to the infraorbital and supraorbital series (Fig. 1a). In other features this specimen is similar to the former.

The jaws of this specimen have been cut clearly revealing the larval features of the buccal cavity of this and the former specimen. Teeth of the premaxillary-maxillary series do not extend posterior to the choanae, whereas in adults they extend posterior to the choanae close to the posterior level of the vomeropalatine series (Fig. 2a). The choanae of adult *Uraeotyphlus* are guarded by a pair of fleshy valve flaps, one lateral and one medial, that lie deep within the choanae but are just visible in palatal view (Fig. 2b). In the larvae the lateral valve flap is present in the adult position, but the medial flap extends into the buccal aperture of the choana and effectively conceals the deeper lateral flap. On its medial side, the medial choanal valve flap is continuous with a large membranous sac filled with a loose fibrous connective tissue. The membranous sac occupies most of the lumen of the choana and displaces the medial valve flap laterally, thereby restricting the buccal aperture of the choana to a narrow slit. The medial valve flap also bears a small but distinctive fleshy medial process (Fig. 2a).

The tongue of larval *Uraeotyphlus* is formed by the anterior margin of the copula that projects into the buccal cavity with a mobile transverse free edge and little intrinsic muscular or glandular tissue. It is thus a primary tongue (Edgeworth, 1935). The fleshy, muscular tongue of the adult (Fig 1b) must form during metamorphosis as the copula disappears, and thus represents a secondary tongue. It appears far less mobile than the larval tongue.

The largest specimen of the series BMNH 82.12.12.12 (TL 95 mm) appears mostly adult in its morphology. There is no remnant of labial folds, the nares have attained a dorsal adult position and the tentacular aperture is much closer to, but not quite yet at, the adult position. Similarly the choanal valves, teeth and tongue have the adult configuration. A tiny subcircular spiracular aperture surrounded by a weak 'gill scar' and lacking the well-developed fleshy flaps of the larvae is present. There are no scales in the annular folds although the skin is distinctly thicker and more glandular than in the larvae. This specimen appears to have nearly completed metamorphosis.

All three specimens have gut contents that include a mixture of organic (chitinous arthropodal) and mineral debris that indicates that they had been actively feeding. In addition, there is no indication of persistent yolk reserves in any of the specimens.

DISCUSSION

It is clear that at least one species of *Uraeotyphlus* has a life history that includes a free-living and self-nourishing larval stage. In the absence of larval specimens of the other species it is not possible to infer their mode of life history with great certainty. It is possible that *U. narayani*, the species studied by Ramaswami (1941), does have direct development, but the small size at which an essentially adult morphology is encountered in this species cannot be considered as strong evidence for the occurrence of direct development because metamorphosis in *U. oxyurus* must occur at a comparably small size. It is more likely that the Uraeotyphlidae, like the 'primitive' families Rhinatrematidae and Ichthyophiidae, is characterised by a larval stage and therefore, until there is positive evidence to the contrary, this is how the family or genus would best be scored if included in any phylogenetic analysis incorporating life history information as character data.

The lack of a fin and lateral compression of the tail in the larvae of *U. oxyurus* is puzzling because these features are found in the larvae of ichthyophiids and rhinatrematids. It is probable that both larval specimens have begun metamorphosis because they are close to the size of the third, and clearly metamorphic, specimen, have no or only faint indications of a lateral line system, and the tentacle has begun to migrate forward to the adult position from the orbit. It is therefore possible that a fin and laterally compressed tail may be present at an earlier, premetamorphic stage of ontogeny. Younger specimens are, however, unknown.

The external nares of most caecilians are dorsolateral. Dorsal nares are a distinctive and probably derived feature of adult *Uraeotyphlus* that are also found in the Scolecomorphidae and several genera of the Caeciliaidae (*Caecilia, Geotrypetes, Hypogeophis, Idiocranium,* and *Oscaecilia*). All these forms with dorsal nares also have anteriorly placed tentacular apertures. The transition from lateral to dorsal nares in *Uraeotyphlus* appears to be associated with the forward ontogenetic migration of tentacle to the anterior adult position. Dorsal migration of the external nares may represent a common epigenetic response in all these forms to the forward migration of the tentacle and

consequent 'crowding' of the rostrum of the snout. This possible epigenetic interaction and lack of independence should be bourne in mind if tentacle and naris positions are to be used as characters for phylogeny estimation.

Adult caecilians typically have subcircular external nares, with the exception of the aquatic or semi-aquatic typhlonectid genera *Nectocaecilia, Potomotyphlus* and *Typhlonectes*. These are the only adult caecilians that have subtriangular nares (Taylor, 1968; Wilkinson, 1989) similar to those seen in larval *Uraeotyphlus*. The aquatic larvae of ichthyophiids and rhinatrematids also have subtriangular external nares that transform into the more typical adult subcircular shape at metamorphosis (pers. obs.) and the same transition is seen in the larvae and adults of the caeciliaid *Sylvacaecilia grandisonae* (Largen *et al.*, 1972). Thus there seems to be a correlation between subtriangular external nares and an aquatic habitus, although the significance of this correlation is not clear. The ontogenetic transformation from subtriangular to subcircular naris shape in 'primitive' caecilians, together with the probability that the subtriangular adult condition is derived within the Typhlonectidae (Wilkinson, 1989) suggests that the derived typhlonectid condition may be paedomorphic. It also provides an example of incongruence between the outgroup and ontogenetic criteria for assessing character state polarities.

The unusual arrangement of the choanal valves of *Uraeotyphlus* which results in a greatly restricted choanal aperture parallels the condition seen in adults of the aquatic typhlonectid *Potomotyphlus*. In this form the valve flaps are also relatively superficial and are partially fused (Nussbaum & Wilkinson, 1989). *Potomotyphlus* is a relatively small headed form that presumably takes prey of restricted size (Wilkinson, 1991). *Uraeotyphlus* larvae are presumably mainly suction feeders and may therefore also be restricted to prey of small size. One speculative explanation of the choanal similarity of *Potomotyphlus* and *Uraeotyphlus* larvae is that the reduction in size of the functional choanal aperture in both forms, whilst accomplished in different ways, may be a common response to the increased problem of prey becoming lodged in the choanae attendant upon the utilization of relatively small prey items. Alternatively, the occlusion of the larval choanal aperture in *Uraeotyphlus* may enhance the efficiency of suction feeding by preventing the flow of water into the buccal cavity through the choanal apertures during rapid buccal expansion.

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EFFECTS OF LOW TEMPERATURE ON TESTICULAR CELLS IN THE MARBLED NEWT, *TRITURUS MARMORATUS* (CAUDATA, SALAMANDRIDAE)

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ABSTRACT

The response of the different germ cell types and glandular tissue of the testis to low temperatures (4°C) and long photoperiods (16L:8D) was studied in the marbled newt (*Triturus marmoratus*) by histologic quantitative methods in the three periods of the annual cycle: quiescence (January-March), germ cell proliferation up to round spermatids (April-June), and spermiogenesis (July-September). Together with each group of cold-exposed newts, another group was maintained at mild temperature (20°C) over the same long photoperiod. At the beginning and end of each period, initial and final wild controls were collected. In the quiescent period, only spermatogonial proliferation was observed in the initial and final controls as well as in the cold-exposed newts. The newts kept at 20°C developed spermatogenesis up to the round spermatid level. At the end of the germ cell proliferation period, the final controls showed round spermatids; the newts exposed to 20°C developed complete spermatogenesis; and the newts maintained at 4°C only presented spermatogonial proliferation. At the end of the spermiogenesis period, the final controls and the newts kept at 20°C showed complete spermatogenesis and developed glandular tissue whereas the newts exposed to 4°C only had round spermatids and had no glandular tissue. Present results suggest that although low temperature does not affect spermatogonium proliferation it impedes both the subsequent steps in spermatogenesis and the development of glandular tissue.

INTRODUCTION

Photoperiod and temperature are the most important external factors controlling the reproductive cycle in amphibians (Galgano & Flachetti, 1940; Cei, 1944; Lofts, 1974). In urodele amphibians inhabiting cold-temperate areas spermatogenesis occurs during spring and summer when the photoperiod is longer and temperature is higher, whereas in autumn and winter, when the photoperiod is short and temperature is low, the testis remains quiescent (Lofts, 1974; Sáez, Fraile & Paniagua, 1990). The duration of the spermatogenesis period varies for the same species depending on the latitude and altitude of the geographic area (van Oordt, 1956; Rouy, 1972). Experiments in several urodele species have shown that 12-16 hr of light per day and temperature of 20°C induce spermatogenesis even during the period of testicular quiescence (Werner, 1969; Steinborn, 1984; Fraile, Paniagua & Rodríguez, 1988). Werner (1969) in *Plethodon cinereus* and Steinborn (1984) in *Triturus cristatus* found that mild temperatures (20-22°C) induce development from spermatogonia into spermatocytes in the newts exposed to short photoperiods (less than 8 hr of light daily). Similar findings were reported by Fraile *et al.* (1988) in marbled newts (*Triturus marmoratus*) kept in complete darkness. However, longer photoperiods (12-16 hr of light) are necessary for obtaining complete spermatogenesis (Werner, 1969; Fraile, Paniagua, Rodríguez & Sáez, 1989a). The effects of moderately low temperatures (10-11°C) have been studied by Werner (1969) and Steinborn (1984). Both authors observed that these temperatures induce spermatocyte formation if the photoperiod is long (12-16 hr), although higher temperatures are required to achieve complete spermatogenesis. These experiments suggest that: (1) neither photoperiod nor temperature controls the initial phase of spermatogenic development; and (2) both long photoperiods and mild temperatures are necessary for meiosis and spermiogenesis.

Nevertheless, these results differ from the data reported by Ifft (1942) who failed to observe development from spermatogonia into spermatocytes in the urodele *Notophthalmus viridescens* maintained at 8°C even when the animals were exposed to long photoperiods. In addition, the experiments on temperature in anurans indicate that mild temperatures are a prerequisite for spermatocyte development (Lofts, 1974; Rastogi, Iela, Sasena & Chiefi, 1976).

These dissimilarities in results might be attributed to differences between species, but also to the period of the cycle in which the animals were exposed, the duration of the exposure and the exact temperature of exposure.

The present study concerns the influence of a temperature of 4°C on each germ cell type and glandular tissue in the testis of the marbled newt in the three different periods of the annual testicular cycle. The temperature was chosen because it was the average temperature during December-January in the area inhabited by the newts. The newts were exposed to the optimal photoperiod according to previous studies (Fraile *et al.*, 1988, 1989a; Fraile, Paniagua, Rodríguez & Sáez, 1989b) and the results were compared with those obtained in newts exposed to the same photoperiod and mild temperature as well as with those obtained in control marbled newts exposed to the environmental temperature and the natural photoperiod in the wild.

MATERIALS AND METHODS

Twenty-four marbled newts (*Triturus marmoratus marmoratus* Latreille) were collected from forested areas in the Province of León (Spain) on December 30th (quiescent period), March 30th (end of the quiescent period), and June 30th (end of the period of germ cell proliferation and meiosis and beginning of the spermiogenesis period) (Sáez *et al.*, 1990). In order to eliminate the influence of body weight in the experimental results, only newts weighing between 9.0 and 9.5 g were selected. The 24 newts captured on each of these days were sorted into three groups of eight animals. One group was killed the next day and used as initial or final controls for the three successive experiments. The other two groups were maintained in the laboratory for three months. Each group was kept in a glass

aquarium (1 x 1 x 0.4 m) containing fresh water up to a depth of 20 cm. A solid surface (a flat-based roughly pyramid-shaped rock) was provided so that the newts could either swim or rest on this surface. Lighting was supplied by cool, white, 14 W fluorescent lamps with a wavelength distribution from 350 to 710 nm. Lighting conditions were maintained at a 16L:8D photoperiod by automatic timers. Each group was kept in a different room provided with a thermostat. Water and air temperatures were maintained at 20±1°C for one group, and at 4±1°C for the other group. The animals received food every two days. To obtain final controls at the end of the spermatogenesis period, eight newts were collected on September 30th and killed the day after.

The animals were weighed, anaesthetized with methanesulphonate, and fixed by perfusion through the aortic cone with the Karnovsky fixative (3% phosphate-buffered glutaraldehyde-paraformaldehyde, mixed in equal proportions) for 30 min. Following this, both testes were removed and weighed. Testicular volumes were calculated by water displacement. The right testes were sliced into small fragments which were embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. The left testes were fixed for an additional 6 hr in the same fixative, dehydrated, and embedded in paraffin. The blocks were used for quantitative studies. Since the wave of germ cell differentiation in the newt testis progresses from the posterior to the anterior pole, only sagittal sections of the whole testis were suitable for quantitative studies. For this purpose, five 6µm-thick sagittal sections of each left testis at points 1/6, 1/3, 1/2, 2/3 and 5/6 of the transverse testicular diameter were selected and stained with haematoxylin and eosin. In each testis, the areas occupied in the five sections by each germ cell type (including their accompanying Sertoli cells and connective tissue cells) and the glandular tissue (developed Leydig cells) were measured with a semi-automatic image analyzer (Kontron, Zeiss, Oberkochen, FRG). The resulting values were divided by the total surface area of the five sections thus obtaining the volume densities of each germ cell type. The absolute volumes per testis for each cell type were obtained by multiplying volume densities by testicular volume and by a correction factor (0.76) which is the result of the transformation of testicular volume after embedding. This factor was previously determined from 50 newt testes by water displacement.

Means and SD for each group of newts were calculated from the values obtained for each animal. Comparison of the means between the different groups in each experiment was carried out by a one-way ANOVA test. For the parameters showing significant differences, comparison between each pair of means was carried out by the two-sample *t*-test.

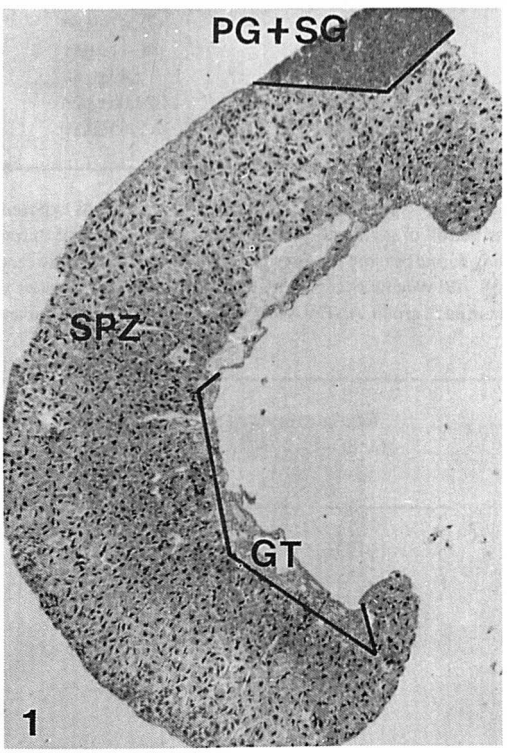

Fig. 1. Testicular lobe from a marbled newt exposed to 40°C and 16L:8D for three months during the period of testicular quiescence. The distribution of germ cell zones is indicated using black lines. PG: primary spermatogonia; SG: secondary spermatogonia; SPZ: spermatozoon bundles; GT: glandular tissue. Haematoxylin and eosin. x15.

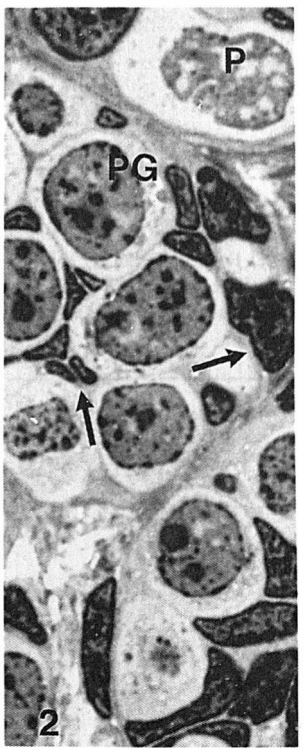

Fig. 2. Primordial germ cells (P) and primary spermatogonia (PG) in the contralateral testis from the same newt. Each primary spermatogonia is completely surrounded by follicular cells (arrows). Toluidine blue. x940.

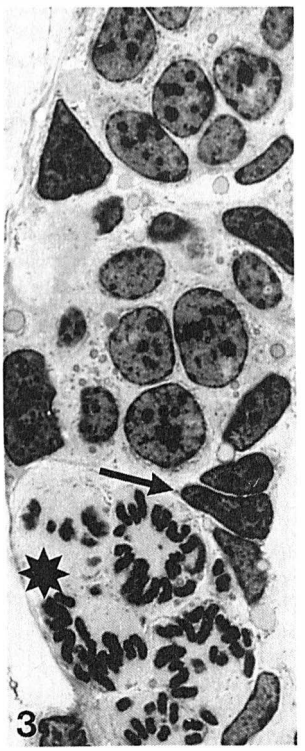

Fig. 3. Zone of secondary spermatogonia in the same testis. The follicular cells (arrow) surrounds groups of secondary spermatogonia. Each cell group is originated from mitoses in primary spermatogonia (star). Toluidine blue. x980.

Weight (body) and volume (testis and germ cells)	Initial controls (Dec. 30)	Final controls (March 30)	Exposure to 16L:8D and 4°C (March 30)	Exposure to 16L:8D and 20°C (March 30)
Body weight	9362±154^{a}	9404±161^{a}	9262±159^{a}	9311±192^{a}
Left testis	90±11^{a}	94±11^{a}	92±9.2^{a}	158±15^{b}
Primary spermatogonia	3.51±0.6^{a}	4.16±0.6^{b}	4.32±0.4^{ba}	3.31±0.3^{a}
Secondary spermatogonia	10.50±1.2^{a}	22.59±2.2^{b}	19.64±3.2^{c}	16.51±2.3^{d}
Primary spermatocytes	-	-	-	33.20±5.5
Round spermatids	-	-	-	59.12±9.8
Elongated spermatids	-	-	-	5.02±1.0
Spermatozoon bundles	67.64±9.3^{a}	59.92±8.1^{a}	61.24±10^{a}	40.22±6.7^{b}
Glandular tissue	8.35±1.2^{a}	7.33±0.7^{a}	6.80±1.0^{a}	0.62±0.1^{b}

TABLE 1. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of testicular quiescence. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weigh and primary spermatogonia.

Weight (body) and volume (testis and germ cells)	Initial controls (March 30)	Final controls (June 30)	Exposure to 16L:8D and 4°C (June 30)	Exposure to 16L:8D and 20°C (June 30)
Body weight	9404±161^{a}	9281±161^{a}	9366±186^{a}	9164±170^{a}
Left testis	94±9.3^{a}	139±16^{a}	75±8.2^{c}	216±18^{d}
Primary spermatogonia	4.16±0.6^{a}	4.15±0.6^{a}	3.76±0.7^{a}	3.81±0.6^{a}
Secondary spermatogonia	22.59±2.2^{a}	37.82±3.9^{a}	23.49±3.1^{ac}	25.23±2.9^{c}
Primary spermatocytes	-	58.57±8.3^{a}	-	78.87±9.8^{b}
Round spermatids	-	37.06±4.2^{a}	-	80.11±10^{b}
Elongated spermatids	-	-	-	2.89±0.4
Spermatozoon bundles	59.92±8.1^{a}	-	39.40±6.2^{b}	23.06±3.2^{c}
Glandular tissue	7.33±0.7^{a}	1.40±0.2^{b}	6.80±1.1.2^{a}	2.03±0.5^{c}

TABLE 2. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of germ cell proliferation and development to round spermatids. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weight and primary spermatogonia.

Weight (body) and volume (testis and germ cells)	Initial controls (March 30)	Final controls (June 30)	Exposure to 16L:8D and 4°C (June 30)	Exposure to 16L:8D and 20°C (June 30)
Body weight	9281±145^{a}	9305±182^{a}	9199±157^{a}	9387±163^{a}
Left testis	139±16^{a}	215±27^{b}	131±16^{a}	210±22^{b}
Primary spermatogonia	4.15±0.6^{a}	3.28±0.7^{b}	3.92±0.4^{a}	5.31±0.8^{c}
Secondary spermatogonia	37.82±3.9^{ab}	43.13±5.1^{c}	38.26±4.3^{bc}	34.41±5.6^{a}
Primary spermatocytes	58.57±8.3^{a}	5.17±0.7^{b}	66.85±7.9^{a}	6.08±0.9^{c}
Round spermatids	37.06±4.2^{a}	8.71±1.1^{b}	21.01±2.9^{c}	9.32±1.3^{b}
Elongated spermatids	-	16.16±2.3^{a}	-	15.71±2.0^{a}
Spermatozoon bundles	-	134.62±18^{a}	-	136.01±18^{a}
Glandular tissue	1.40±0.2^{a}	3.93±0.5^{b}	0.96±0.4^{c}	3.16±0.5^{d}

TABLE 3. Body weight (mg), left testis volume (mm^{3}), and volume occupied by each germ cell type and glandular tissue (mm^{3}) in newts exposed to natural or long photoperiods and low or mild temperatures for three months during the period of spermiogenesis. Values are expressed as means ± SD. For each parameter, the values coinciding in one or more superscript letters do not differ significantly; and the values with completely different superscript letters differ significantly (P<0.05). The volume occupied by each germ cell type includes that occupied by their accompanying follicular and interstitial cells. The sample size was eight in each group. ANOVA test was significant for all parameters except for body weight and secondary spermatogonia.

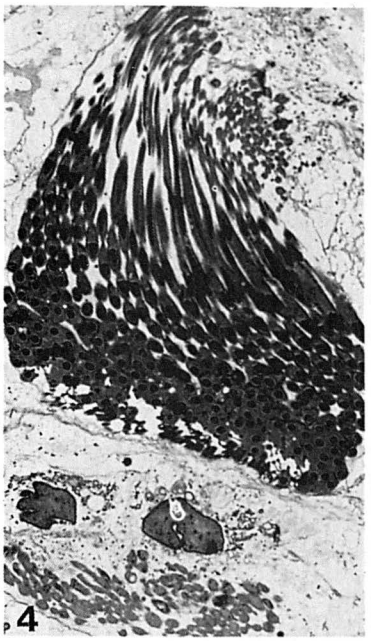

Fig. 4. Spermatozoon bundle in the same testis. Toluidine blue. x580.

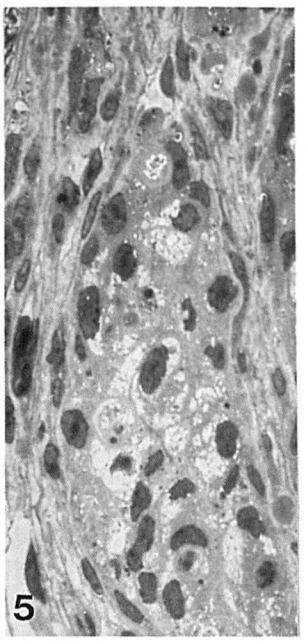

Fig. 5 Glandular tissue in the same testis. Toluidine blue. x365.

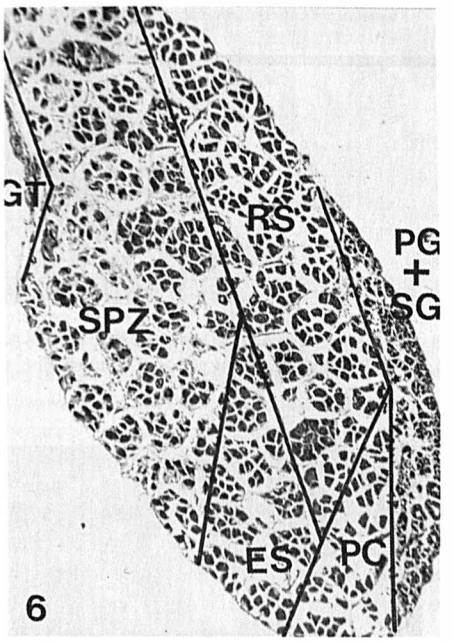

Fig. 6. Testicular lobe from a marbled newt exposed to 20°C and 16L:8D for three months during the quiescent period. The distribution of germ cell zones is indicated by black lines. PC: primary spermatocytes; RS: round spermatids; ES: elongated spermatids; for the other letters see fig. 1. Haematoxylin and eosin. x22.

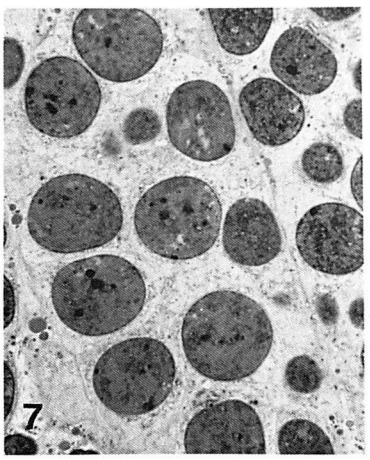

Fig. 7. Zone of primary spermatocytes in the contralateral testis from the same newt. Toluidine blue. x700.

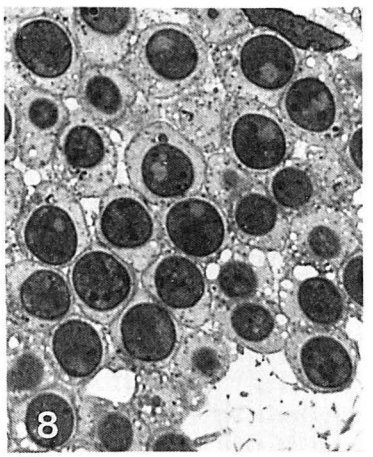

Fig. 8. Zone of round spermatids in the same testis. Toluidine blue. x790.

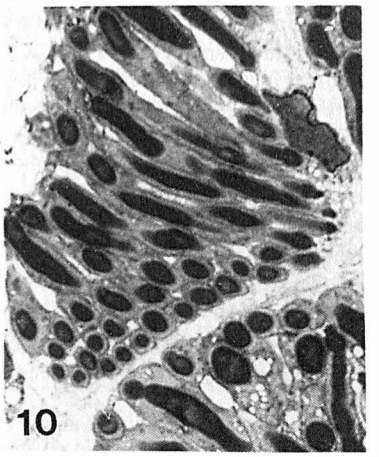

Fig. 10. Elongated spermatids in the same testis. Toluidine blue. x770.

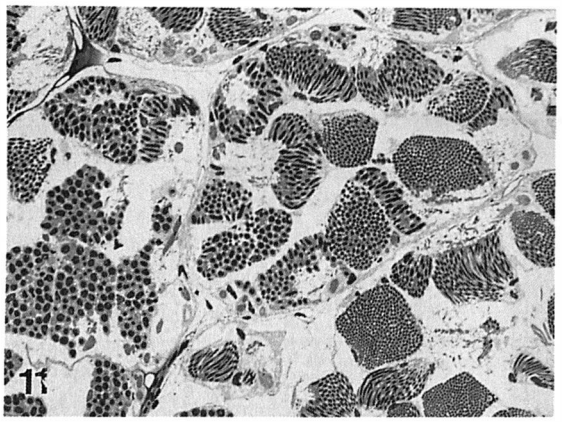

Fig. 11. Spermiogenesis in the testis of a final control newt sacrificed at the end of the spermiogenesis period. Toluidine blue. x115.

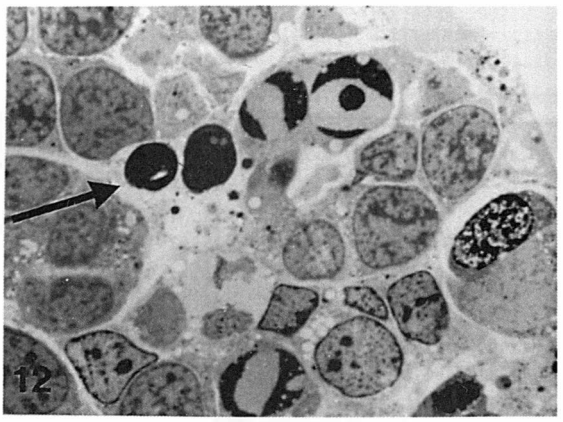

Fig. 12. Degenerating (arrow) spermatocytes in the testis of a marbled newt maintained at 4°C and 16L:8D for three months during the period of spermiogenesis. Toluidine blue. x630.

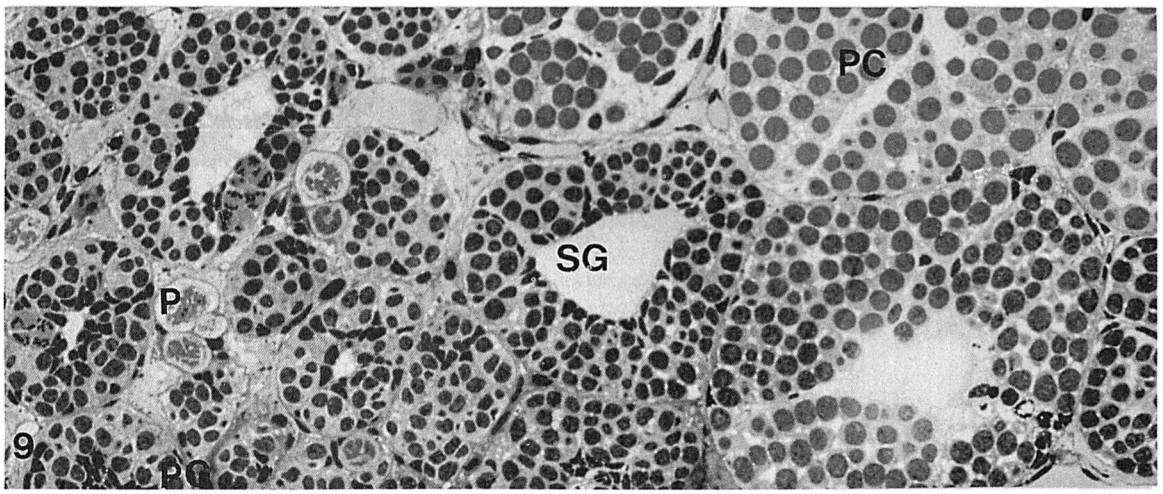

Fig. 9. Part of a testicular lobe from a final control newt sacrificed at the end of the germ cell proliferation period showing primordial germ cells (P), primary (PG) and secondary (SG) spermatogonia, and spermatocytes (PC). Toluidine blue. x200.

RESULTS

Period of Testicular Quiescence (January-March)

No significant differences between the initial controls, final controls, and newts maintained at 4°C were found for any of the parameters measured (Table 1). No spermatogenesis occurred in these newts (Fig. 1). The germ cell types present were primordial germ cells, primary (Fig. 2) and secondary spermatogonia (Fig. 3) and spermatozoon bundles (Fig. 4), these and the glandular tissue (Fig. 5) were probably formed in the preceding cycle (Sáez et al., 1990). The newts maintained at 20°C showed larger testes and developed spermatogenesis up to the level of round spermatids and a few elongated spermatids (Figs. 6-8). The volume occupied by the glandular tissue and that occupied by spermatozoon bundles were lower than in the other groups, suggesting a certain level of spermatozoon release (Table 1).

Period of Germ Cell Proliferation up to Round Spermatids (April-June)

The last controls presented larger testes than the first ones, germ cell development up to round spermatids level, and scanty glandular tissue (Fig. 9). Spermatogenic development was more marked in the newts exposed to 20°C (Table 2). These animals had elongated spermatids (Fig. 10) and their spermatozoon bundles were probably formed during the experiment. The testes of the newts exposed to 4°C were similar to those of the first controls although the former testes were smaller in size and contained less spermatozoon bundles (Table 2).

Period of Spermiogenesis (July-September)

The final control as well as the newts exposed to 20°C achieved spermatogenesis (Fig. 11) and their glandular tissue was increased. The newts exposed to 4°C did not develop spermiogenesis and were similar to the initial controls except for a decrease in the volume occupied by the round spermatids (Table 3). Vacuolated cells with pyknotic nuclei corresponding to degenerating primary spermatocytes or round spermatids could be observed in the newts kept at 4°C (Fig. 12).

DISCUSSION

The results of this study indicate that, during the phase of testicular quiescence, long photoperiod failed to induce spermatocyte formation at cold temperatures (4°C). Since spermatocytes develop in marbled newts (Fraile *et al.*, 1988)

and several other urodele species when they are exposed to short photoperiods and mild temperatures (20°C) during this phase of the cycle (Werner, 1969; Steinborn, 1984), it would seem that temperature, and not photoperiod, induces spermatocyte formation in these urodeles. The observation of spermatocytes in several urodele species maintained at 10-11°C and short photoperiods during the quiescent phase (Werner, 1969; Steinborn, 1984) can be explained on the basis that these temperatures (but not 4°C) are permissive to spermatocyte formation.

Present results show that a temperature of 4°C did not hinder proliferation of secondary spermatogonia during testicular quiescence. In the anuran *Rana esculenta* (Rastogi *et al.*, 1976) and in other anuran species (Lofts, 1974) the environmental winter temperature favours the development of primary spermatogonia while secondary spermatogonia only develop with the mild temperatures in spring.

The effect of temperature on spermatogenic development in amphibians seems to be mediated by the hypothalamic-hypophyseal system (Mazzi, 1970).In the anuran *R. esculenta*, the absence of spermatocyte formation at low temperatures seems to be caused by the suppression of gonadotropin secretion by the hypophysis and not by decreased activity of gonadotropin receptors in the germinal epithelium. In this species the germinal epithelium is sensitive to gonadotropin stimulation at wide ranges of temperature since gonadotropin administration induces spermatocyte formation at either low or high temperatures (Rastogi *et al.*, 1976). In another anuran species, *Rana temporaria*, low temperatures only insensitize the germinal epithelium to gonadotropins during the first part of the quiescent period (van Oordt, 1956; van Oordt & Lofts, 1963). In other anurans (Lofts, 1974) and in the urodele *T. cristatus* (Galgano & Flachetti, 1940) low temperatures inhibit the sensitivity of the germinal epithelium at any time of the year. This latter probably occurs also in the marbled newt and other urodele species; in the red-bellied newt, gonadotropin levels are as high or even higher in the period of quiescence (with low environmental temperatures) as in the period of germ cell proliferation (with mild temperatures) (Tanaka, Hanaoka & Takikawa, 1980; Tanaka, Takikawa & Wakabayashi, 1981). Studies on follicle-stimulating hormone (FSH) receptors in the urodeles *Cynops pyrrhogaster* (Kubokawa & Ishii, 1980) and *Hynobius retardatus* (Kubokawa, Moriya & Ishii, 1985) indicated that the gonadotropin affinity of FSH receptors in these urodeles decreases with low temperature (0°C).

In the annual cycle of the marbled newt, spermatozoon bundles are released from the testis at the beginning of the period of germ cell proliferation (Sáez *et al.*, 1990). The testes of newts exposed to 4°C during the period of germ cell proliferation show the same absence of germ cell development as the initial controls although a certain degree of spermatozoon release occurred. This suggest that spermatozoon release is not secondary to spermatocyte formation. Both temperature and photoperiod might be important factors controlling this process. Ifft (1942) suggested that temperature and not photoperiod controls this process, as spermatozoon release was not observed in the urodele *Notophthalmus viridescens* maintained at 8°C and either total darkness or constant light during the period of cell proliferation. This finding agrees with our previous observation of spermatozoon release in marbled newts exposed to mild temperature (20°C) and short photoperiods during this phase of the cycle (Fraile *et al.*, 1989a). However, in marbled newts maintained at 20°C during the quiescent period, spermatozoon release only occurred in the animals exposed to long photoperiods and only to a certain degree (Fraile *et al.*, 1988). All these findings lead us to suggest that, in addition to temperature, other factors such as photoperiod, a certain degree of spermatogonial proliferation, and the persistence of the glandular tissue are probably involved.

In the testicular cycle of the marbled newt, a pronounced reduction in glandular tissue associated with spermatozoon release is observed in the period of germ cell proliferation. Spermatozoon release seems to be regulated by a decrease in androgen production and an increase in gonadotropin secretion (Lofts, 1974). Hormone studies on the annual testicular cycle of the newt *Cynops pyrrhogaster* (Tanaka & Takikawa, 1983) indicated that plasma testosterone levels decrease abruptly in spring when spermatocyte proliferation begins. In our study, glandular tissue persisted in the newts exposed to 4°C during this period. However, this does not mean that androgen levels remain high because the abundant glandular tissue present in winter in the natural annual cycle is inactivated by the low temperatures (Tanaka & Takikawa, 1983; Fraile, Paniagua, Sáez & Rodríguez, 1989c; Fraile, Paniagua, Rodríguez, Sáez & Jiménez, 1989d). Therefore, present results suggest that cold temperatures hinder the disappearance of the glandular tissue and inactivate androgen synthesis by this tissue that would otherwise lead to spermatozoon release.

In the marbled newt, exposure to 4°C during the spermiogenesis period impedes spermiogenesis and causes degeneration of spermatids and spermatocytes. This effect seems to be related with temperature and not with photoperiod since spermiogenesis occurs in newts exposed to mild temperatures and either short or long photoperiods (Fraile *et al.*, 1989b). Therefore, temperature seems to regulate not only spermatocyte formation and meiosis but also spermiogenesis. In previous experiments we found that high temperatures (30°C) do not affect spermatocyte formation and meiosis although they hinder spermiogenesis (Fraile *et al.*, 1989). The negative effects of cold temperatures on spermiogenesis might be mediated by androgen secretion. In mammals (Ritzén, Biotani, Parvinen, French & Feldman, 1982; Parvinen & Ruokonen, 1982; Paniagua, Rodríguez, Nistal, Fraile, Amat & Regadera, 1986) and in some anuran species (Rastogi, Tammaro, di Meglio, Iela, di Matteo & Chieffi, 1981) spermatid differentiation is a directly androgen-dependent process. In many urodeles species (Ucci, 1982; Pudney & Callard, 1984; Lecoteaux, Garnier, Bassez, Joly, 1985) including the marbled newt (Fraile et al., 1990), spermatozoon formation precedes glandular tissue formation and the subsequent increase in androgen synthesis. Therefore, spermiogenesis in urodeles does not seem to be androgen-dependent although it might be mediated by the increase in the gonadotropin levels observed at the beginning of the spermatogenesis period (Tanaka *et al.*, 1980). As has been said for spermatocyte formation, the negative effects of low temperatures on spermiogenesis in the marbled newt might be related with spermatid insensitivity to gonadotropins.

At the end of the spermiogenesis period the glandular tissue increases again in the marbled newts exposed to mild temperatures but not in the animals maintained at 4°C. In previous experiments we have shown that photoperiod does not influence glandular tissue development in the marbled newt (Fraile *et al.*, 1989b) while high temperatures hinder glandular tissue formation (Fraile *et al.*, 1989c). In many urodele species (Specker &

Moore, 1980; Tanaka & Takikawa, 1983; Imai, Tanaka & Takikawa, 1985; Fraile *et al.*, 1989d) the favourable temperatures for testosterone synthesis are comprised within a narrow range (the mild temperatures of early spring and late autumn). However, the role of cold temperatures in the lack of glandular tissue development in the spermiogenesis period might be secondary to the lack of spermiogenesis since transformation of interstitial peritubular cells into Leydig cells only occurs in the cysts in which spermiogenesis is completed (Fraile *et al.*, 1990).

The results of the present and previous studies on external factors controlling testicular function in newts suggest: (1) neither temperature nor photoperiod is necessary for spermatogonial proliferation; (2) mild or high (up to 30°) temperatures are a necessary and sufficient condition for spermatocyte formation; long photoperiods are not necessary; (3) these temperature are a necessary but insufficient condition for meiosis; long photoperiods are also necessary; and development of the glandular tissue; high temperatures hinder these processes while photoperiod has no influence.

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FEEDING AND DIGESTION IN THE OMNIVOROUS ESTUARINE TURTLE *BATAGUR BASKA* (GRAY)

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ABSTRACT

The emydid river terrapin *Batagur baska* (colloquially known as the tuntong) lives in rivers and estuaries of S.E. Asia. The species is omnivorous, but predominantly herbivorous from the hatchling stage onwards. Young river terrapins (3-4 months; 140-200 g body wt) from a headstarting programme in western Malaysia were studied. Appetite on a plant diet (kangkong; *Ipomoea aquatica:* Convolvulacea) was extremely high (16% body wt d^{-1} on fresh wt basis); river terrapins spend long periods of browsing, using the double serrations of the upper beak to cut up plant material. The serrations also function in ratchet like fashion to allow large leaves to be progressively moved into the oesophagus without the turtle losing contact with the food. *Batagur baska* readily eats water hyacinth (*Echornia crassipes*), a plant which often chokes tropical waterways.

River terrapins fed on trash fish move a meal through the gut more quickly (total gut clearance time, TGCT = 5 days) than do those fed upon kangkong (TGCT = 6 days). The gut features a large stomach, a small intestine of moderate length but large diameter and a capacious large intestine. The gut does not sort material. Assimilation efficiency on a diet of fish (mean assimilation of dry mass = 91.6%, of energy (joules) = 90.5%, of protein = 97.4%) is much greater than on a diet of kangkong (43.2%, 38.6% and 66.0% respectively). It is recommended that headstarted animals are regularly fed on fish to improve growth rates. River terrapins readily eat plant material in salinities between 0 and 19.8‰, but refuse to eat in water of 23.1‰ or more, presumably to avoid the incidental drinking of water with a higher ionic content than their blood.

INTRODUCTION

The river terrapin, *Batagur baska*, is a large emydid turtle (< 25 kg, 59 cm carapace length) once common in the lower reaches of the large river systems of S.E. Asia, but now much reduced in numbers. Known as tuntong by Malays (though this term is also applied to the related *Callagur*), river terrapins (Iverson, 1985) have also been referred to as batagurs in the older scientific literature. River terrapins are amongst the few reptiles that exploit the brackish waters of estuaries. Outside the breeding season they often forage in estuaries and mangroves (Maxwell, 1911; Moll, 1978) and are occasionally seen in sea water (Gunther, 1864), though Davenport & Wong (1986) demonstrated that their physiological ability to cope with a saline environment is limited, and that river terrapins largely survive by deploying behavioral osmoregulatory responses.

Batagur baska is an endangered species, its numbers having fallen by a combination of habitat loss and direct exploitation of adults and eggs for human food. Headstart programmes were established in Malaysia in the early 1970s and have been described by Moll (1980) in his comprehensive study of the biology of the species.

River terrapins are true omnivores and readily eat invertebrates, fish or carrion as well as plant material. However, from the study of Moll (1980) it appears that they are predominantly herbivorous, browsing upon floating and riverside vegetation as well as eating fruit (including the fruit of mangrove trees). He found that 45% of the volume of faecal samples was composed of the remains of leaves and stems, 25% of fruit (overwhelmingly mangrove fruit) and 30% of mollusc shells (pelecepods of unidentified species). Before their numbers were reduced by human exploitation river terrapins were probably of considerable importance ecologically since they break up and recycle large quantities of plant material.

Many aquatic chelonians are omnivores, but in early life most of such species are characterised by predominantly carnivorous feeding (e.g. green sea turtles, *Chelonia mydas* (Hirth, 1971; Booth & Peters, 1972), the freshwater pond slider *Trachemys scripta* (Clark & Gibbons, 1969)) and it seems that a high protein diet is needed to sustain the high growth rate of hatchlings and juveniles. Only river terrapins, the pleurodiran river turtles of South and Central America (*Podocnemys sp.*) and *Dermatemys* appear to be able to grow quickly from the earliest stages on a diet rich in plant material. The first objective of the study reported here was to determine whether tuntong are particularly efficient in assimilating nutrients from vegetation, or simply process unusually large quantities of food. A second objective was to compare food processing and assimilation in river terrapins fed either on plant or fish diets. Thirdly, because river terrapins spend much of their time in estuaries, especially mangroves, but are known not to feed when held in sea water (Davenport & Wong, 1986), it was decided that the effect of environmental salinity on feeding should be investigated. The final task was to observe and analyse the feeding mechanism of river terrapins, particularly in *Batagur baska* browsing on floating vegetation - a challenging proposition from a biomechanical point of view.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Twenty young (3-4 months; 140-200 g body weight) river terrapins were borrowed from a hatchery/headstart station at Sungar Pinang near Alor Setar, N.W. Malaysia. Individual animals were identified by numbering the carapace with typewriter

correction fluid. The river terrapins were held in fresh water at the temperature employed throughout this study (30±2°C) and fed daily upon kangkong (*Ipomoea aquatica* Forsk : Family Convolvulacea) until used in experiments. Kangkong was used as the basic food for several reasons. First, although widely eaten as an inexpensive vegetable in Malaysia, kangkong grows wild, especially by the side of rivers. It is therefore a likely food item of wild river terrapins, though not specifically mentioned by Moll (1980) who was unable to identify the leaves and stems whose remains made up 45% of faecal volume in Perak river specimens. Secondly, kangkong is the main item of food fed to river terrapins reared at the two river terrapins hatcheries in Malaysia, at Sungar Pinang and at Bota Kanan in the state of Perak. Hatchling and juvenile river terrapins are now fed almost exclusively on kangkong, with only occasional supplementation with banana. This contrasts with earlier reports by Moll (1980) that they were regularly fed on fish. The hatcheries still feed older animals (5-7 years old) on a mixed diet of kangkong and trash fish (i.e. fish of no commercial value or undersized commercial fish - species unknown). Finally, pilot experiments revealed that kangkong maintains a constant weight when held in fresh water for periods of many hours, thus allowing accurate estimates of appetite (see below).

Feeding Behaviour

Four river terrapins were held for 24 hr without food and then placed in a large, well lit, glass aquarium marked out on three sides and the bottom with a 1 cm inked grid. The subsequent actions were videorecorded with a Panasonic camera and videorecorder (AG6200). The camera incorporated a 0.001 s shutter, allowing blur-free field-by-field analysis. The river terrapins were offered two food items; kangkong and water hyacinth (*Echornia crassipes*). To supplement the videotape data, 35 mm photographs and drawings of the jaws of the turtles were made. Animals feeding on trash fish were observed but not filmed.

Effect of Salinity on Feeding

Two animals were placed in each of 11 aquarium tanks containing water of the following salinities: 0, 3.3, 6.6, 9.9, 13.2, 16.5, 19.8, 23.1, 26.4, 29.7, and 33‰. The animals were allowed to settle down for one hour and were then offered intact leaves of kangkong (5 leaves per aquarium). The tanks were inspected after 1, 6 and 24 hr and the number of leaves consumed (or showing evidence of bites) counted.

Measurement of Appetite

The satiation ration for river terrapins eating kangkong was assessed in the following manner. Three animals were deprived of food for 48 hr and then each was offered a preweighed 'meal' of kangkong leaves (not stems as they hold liquid water and cannot be weighed accurately) in a separate aquarium. Weighing of kangkong was performed after drying in paper towels. Each animal was left with its 'meal' for 24 hr, then all remaining pieces of plant material were collected and reweighed. In pilot experiments it had been established that 50 g of kangkong could be weighed repeatedly with an accuracy of 0.2-0.5 g over 24 hr periods. Several examples of kangkong were weighed and dried to constant weight in an oven at 40°C. The mean water content of kangkong was 93% by weight. When eating vegetation, river terrapins are browsers, often feeding intermittently for several hours before satiation; this explains the rather unusual method of assessing appetite.

Gut Transit

Movement of food through the gut was studied by monitoring the progress of chromic oxide-labelled meals, and by X-radiography. Firstly, total gut clearance times (TGCTs) were established. Three river terrapins were fed daily on trash fish for seven days ('fish diet'); three other turtles were fed on kangkong for a similar period ('plant diet'). Each group was then fed on an appropriate single chromic oxide-labelled meal followed at daily intervals by normal unlabelled meals. Terrapins on the fish diet were fed a labelled meal made up by mincing 100 g trash fish fillets mixed with 5 g chromic oxide. The meal was bound together with agar. River terrapins eating the plant diet were fed on an agar-bound mixture of minced kangkong (100 g) and chromic oxide (10 g). The turtles were held in separate tanks which were inspected daily for the presence of chromic oxide-labelled faeces. During each inspection faeces were broken up and inspected with a low power binocular microscope.

X-radiography was performed on two river terrapins which had previously been fed on kangkong for more than two weeks. Each was deprived of food for 48 hr and then offered a meal made up in the following fashion. 100 g kangkong was finely minced and thoroughly mixed with 2 g barium sulphate powder. A few hundred barium/polystyrene spheroids (1 mm diameter; ICI Ltd) were stirred into the mixture, followed by several thousand lead glass beads (ca. 0.1 mm diameter). Warm agar solution was added and the mixture stirred until it began to set (thus preventing settlement of the spheroids and beads). When setting was complete the food was cut into pieces and offered to the river terrapins. Both ate voraciously for about 10 min. Feeding intensity then fell and the animals were transferred to another tank which contained floating kangkong. The animals were X-rayed at the following intervals after the labelled meals: 1, 7, 25, 31, 49 and 97 hr. Fresh kangkong was available to the turtles throughout this period and faeces were removed several times per day to avoid reingestion of labelled material.

Absorption/Assimilation Efficiency

To determine the efficiency of absorption of energy and protein in river terrapins fed upon plant and animal diets, two groups of three turtles were fed exclusively on one or other diet for two weeks. They were then fed for eight days on chromic oxide-labelled food (prepared as described above for gut transit experiments). Samples of food and freshly-voided faeces were then collected from each animal, frozen, freeze-dried and stored in a freezer at -25°C until analysed. Chromic oxide content was measured by the method of McGinnis & Kasting (1964). Energy content was determined by the wet oxidation method of Ivlev (1935); appropriate corrections for unreacted protein were applied to the data. Protein content was estimated by measuring total nitrogen (micro-Kjeldahl technique).

RESULTS

Feeding Behaviour

When feeding on trash fish the behaviour of river terrapins was similar to that of other emydid turtles. Small pieces of flesh were swallowed whole; large items were torn apart by combined action of jaws and forelimbs. Typically a turtle would bite

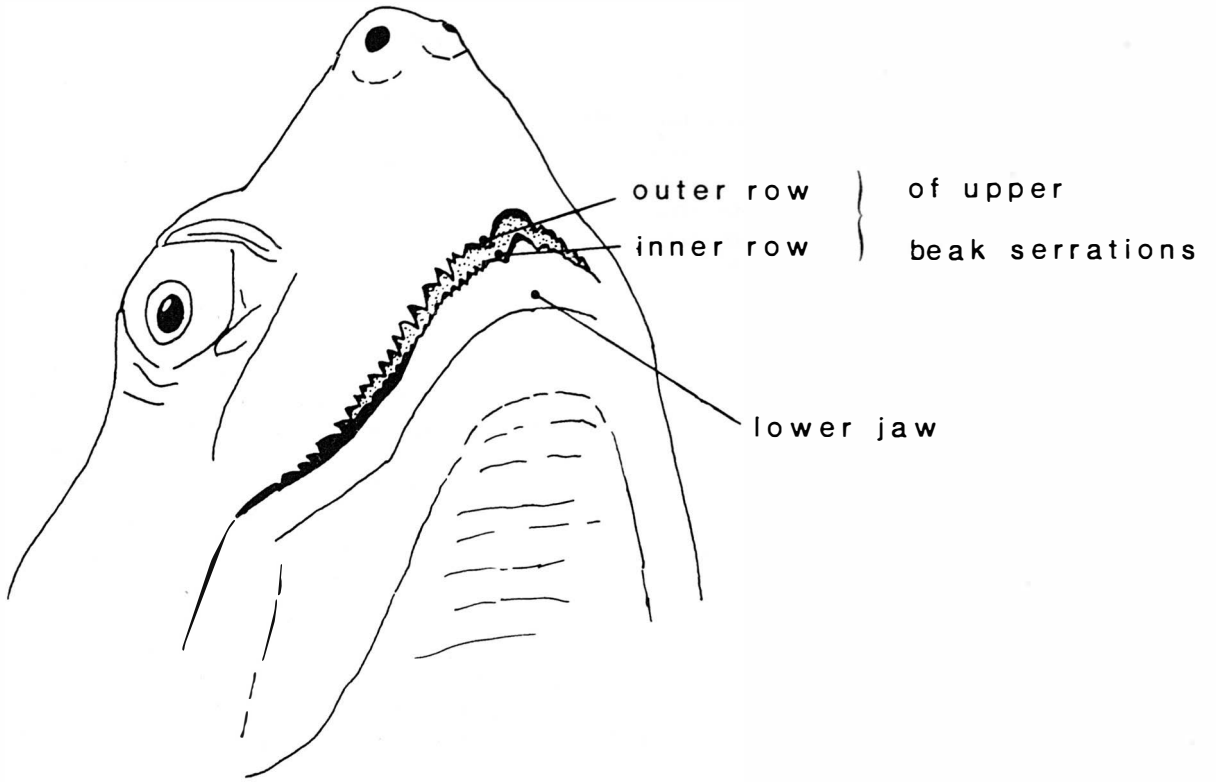

Fig. 1. Drawing of head of young *Batagur baska* to show serrations of beak. Note that the two visible rows of serrations are both on the upper jaw in this view (i.e. the stippled area is the lateral surface of the inner row of serrations). Because the jaws are firmly closed the edge of the lower beak is not visible as it is overlapped by the twin rows of serrations on the upper jaw. A *camera lucida* drawing made from a 35 mm photographic slide.

into a piece of fish with the beak and then push the food item forwards by simultaneous action of the forelimbs - until the piece of fish broke to leave a morsel in the mouth. At the onset of this pushing the forelimbs were extended and rotated medially so that the plantar surfaces of the forefeet were in contact with the prey, and the two sets of claws directed towards one another. River terrapins have extensively webbed forelimbs and relatively small claws. The claws appear to give purchase on prey items rather than being actively used to shred tissue.

When feeding on plant material, the beak structure of the river terrapins assumed great importance. From Fig. 1 it may be seen that the upper jaw is lined by a double row of recurved serrations which are more pronounced anteriorly. At the front of the upper jaw the two most medial pairs of inner and outer serrations are separated by curved central gaps. Serrations on the lower jaw are rather less pronounced, but anteriorly, in the midline, the lower jaw features a pronounced, curved and sharp hook which fits inside the gap in the serrations of the upper beak when the jaws are closed. When eat-

	Elapsed time (h)		
Salinity ‰	1	6	24
0.0	5	-	-
3.3	5	-	-
6.6	5	-	-
9.9	5	-	-
13.2	1	5	-
16.5	5	-	-
19.8	5	-	-
23.1	0	0	0
26.4	0	0	0
29.7	0	0	0
33.0	0	0	0

TABLE 1. Effect of salinity on feeding in river terrapins. Data show number of kangkong leaves (out of 5) showing evidence of browsing.

	Time after meal labelled with chromic oxide (days)						
	1	2	3	4	5	6	7
Plant diet							
Animal 1	-	-	-	xx	x	x	-
2	-	-	-	xx	x	x	-
3	-	-	-	xx	x	x	-
Fish diet							
Animal 4	-	-	xx	xxx	x	-	-
5	-	-	xx	xxx	x	-	-
6	-	-	x	xxx	x	-	-

x = trace quantities of label in faeces (only visible by microscopic inspection)
xx = noticeable quantities of green label
xxx = copious labelled faeces
- = no label in faeces

TABLE 2. Gut transit times of contrasting meals in young *Batagur baska* at 30°C.

	Observed no. of radio-opaque particles					
Hours after labelled meal	1 hr	17 hr	25 hr	31 hr	49 hr	97 hr
I. Gut section						
A. Stomach						
barium spheroids	34	30	3	-	-	-
glass beads	103	93	3	-	-	-
B. Small Intestine						
barium spheroids	-	4	29	20	4	-
glass beads	-	10	100	68	6	-
C. Large Intestine						
barium spheroids	-	-	2	6	22	-
glass beads	-	-	-	10	62	-
II. Defecated						
barium spheroids	-	-	-	8	8	34
glass beads	-	-	-	25	25	103

TABLE 3. Progress of radio-opaque material along the gut of young *Batagur baska* fed on kangkong.

	Assimilation rate (%)		
	dry mass	energy (joules)	protein
Plant diet (n=3)			
mean	43.2	38.6	66.0
S.D.	23.9	21.1	17.9
Fish diet (n=3)			
mean	91.6	90.5	97.4
S.D.	1.5	0.9	0.9

N.B. For all three nutrient categories, a t-test indicates that there was a significant difference between mean assimilation efficiencies on plant and fish diets (P<0.05).

TABLE 4. Assimilation of nutrients from contrasting diets in young *Batagur baska* at 30°C.

Nutrient	A. Dry weight basis		B. Wet weight basis	
	1. kangkong	2. fish	1. kangkong	2. fish
	93% water	80% water	93% water	80% water
protein (mg g^{-1})	276	583	19.3	117
energy (Kjoules g^{-1})	27.6	25.8	1.9	5.2

TABLE 5. Protein and energy content of kangkong and trash fish.

ing plant material the terrapin used the limbs to achieve a stable shell position in the water column, so that the head, substantially retracted, was very close to the food item. The turtle then struck at the plant, simultaneously extending the neck and gaping the jaws widely. As the jaws snapped shut, the floor of the buccal cavity was depressed so that the food was sucked into the mouth. In some cases the sharp serrations sliced through the leaf structure cleanly and the portion of plant material was then swallowed. On other occasions, particularly when eating long kangkong leaves, the river terrapins used the beak serrations in a ratchet-like fashion, repeatedly striking and swallowing the plant material, but without breaking pieces off, so that the whole leaf was progressively moved into the oesophagus, the beak serrations stopping material escaping between bites. When feeding on water hyacinth, the river terrapins climbed onto the raft of floating plants and browsed continuously for periods of up to 30 min. The finding that river terrapins will readily eat water hyacinth is interesting as Moll (1980) reported that specimens of the species were often found in association with water hyacinth, but he was unable to confirm that they ate it.

Effect of Salinity on Feeding

From the data shown in Table 1 it is evident that river terrapins fed readily at salinities of 19.8‰ and below, but completely refused to feed at salinities of 23‰ and above.

Appetite

Young river terrapins (145-192 g body wt) consumed considerable quantities of plant material on a fresh weight basis (mean±SD = 15.9±0.42% body wt d^{-1}). Even when allowance is made for the high water content of kangkong (93%), the mean feeding rate is equivalent to 11.5 g dry matter per kg live body weight per day - more than three times the ingestion rate reported for a range of herbivorous/omnivorous marine, freshwater and terrestrial turtles by Bjorndal (1985).

Gut Transit

Total gut clearance time (TGCT) for river terrapins fed on a diet of kangkong was 6 days (see Table 2), although most labelled faeces were voided on the 4th day (the first day on which labelled faeces were seen). TGCT on a trash fish diet was shorter, the first labelled faeces being seen on day 3 and all animals having cleared the gut of label within 5 days.

X-radiography was successful in the case of one of the two animals studied (the other vomited part of its meal, leaving too little material for analysis). The first X-radiograph, taken 1 hr after the meal, showed that all of the meal was contained within the stomach and that the animal had swallowed 34 barium spheroids and 103 glass beads as well as the barium-labelled

kangkong. From Table 3 it may be seen that material had started to move out of the stomach after 7 hr. The stomach image was much reduced in size, indicating that some fluid reabsorption had taken place. After 25 hr there was still some material in the stomach, but most had moved into the small intestine. After 49 hr most labelled material was in the large intestine or had been defecated. All label had disappeared by the time 97 hr had elapsed. The transit time recorded with the radio-opaque diet was significantly shorter than for turtles given chromic oxide labelled kangkong. Two factors may have caused this; the 48 hr period of food deprivation before the radio-opaque meal and frequent handling for X-radiography.

The X-ray images showed that river terrapins do not exhibit prolonged oesophageal storage of food, unlike the carnivorous emydid *Mauremys caspica* (Davenport & Kjõrsvik, 1988). The stomach is large and there is no evidence of a powerful pyloric sphincter. The small intestine is of moderate length, but is of much wider diameter than in carnivorous turtles. The large intestine is fairly short, but capacious. There was no evidence of sorting by the gut; spheroids, glass beads and barium powder all moved along the gut together.

Absorption/Assimilation Efficiency

Assimilation efficiencies were calculated as described by Maynard & Loosli (1969) and are presented in Table 4. Despite the shorter gut transit time on a diet of trash fish, it may be seen that nutrients were absorbed from this diet with much greater efficiency than from the diet of kangkong; the difference is statistically significant (*t*-test for small samples). Table 5 gives the protein and energy content of the two diets. On a dry weight basis kangkong has a similar energy content to trash fish, but less than half the protein. However, on a wet weight basis the discrepancy between the diets is much greater, with the trash fish containing 6.1 times as much protein and 2.7 times as many joules.

DISCUSSION

The lower reaches of the river systems of S.E. Asia are enormously productive habitats. Input of energy from the forests surrounding the rivers is great (in the form of leaves, fruit, flowers etc.), but is matched in the tidal parts of the systems by the primary productivity of the marshes and mangroves, benthic algae and epiphytes. There is a superabundance of organic material, much of which is exported to the neighbouring continental shelf in the form of fish, invertebrates and detritus. Estuaries of this type are emphatically not food-limited ecosystems; predation and abiotic environmental influences (particularly salinity) are more important controllers of populations. From the data presented in this study it would seem that river terrapins eat unusually large quantities of plant material which pass through the gut in about the same time (6 days) as in young green turteles, *Chelonia mydas* (Davenport *et al.*, 1989), although comparisons of this type are difficult because of difference in holding temperature. Little information is available about protein assimilation rates in herbivorous reptiles, but the mean rate of assimilation of energy from kangkong by river terrapins (38.6%) is within the range reported for green turtles feeding on sea grass (Bjorndal, 1980) and similar to that reported for giant Aldabran tortoises (*Geochelone gigantea*) eating terrestrial vegetation by Hamilton & Coe (1982), i.e. 34.5%. Zimmerman & Tracy (1989) have summarized energy assimilation data for a range of herbivorous and carnivorous reptiles (especially lizards). These workers reported that the energy assimilation efficiency of herbivorous Testudinata ranged between 34 and 69%. There is therefore no indication that river terrapins are especially efficient at assimilating material from a plant diet; they fuel their growth by voracious feeding on a virtually unlimited resource. In this respect their nutritional physiology is consistent with the hypothesis of Sibly (1981), that animals in a food-limited ecosystem need to maximise assimilation efficiency to get the most out of units of food, whereas animals in a food-rich ecosystem should maximise ingestion rate and process food relatively quickly.

When feeding on trash fish, river terrapins show very high assimilation efficiencies (mean values of 91.6% for dry mass, 90.5% for energy and 97.4% for protein), similar to those exhibited by estuarine crocodiles, *Crocodylus porosus* (Davenport *et al.*, 1990). Few data for carnivorous emydid turtles are available and again the problem of comparability arises because of different thermal conditions, but Kepenis & McManus (1974) reported an assimilation rate (joules) of 80% for young painted turtles (*Trachemys picta*), while Davenport & Kjõrsvik (1988) recorded an assimilation efficiency for energy of 46% in adult Caspian terrapins, *Mauremys caspica*. This finding, that an omnivorous species can be more efficient in assimilating energy and protein from a diet of fish than its carnivorous relatives was unexpected. However, the total gut clearance time for *Batagur baska* fed on fish (TGCT=120 hr) is much longer than those reported for *Trachemys* (TGCT=59 hr) or *Mauremys* (TGCT=72 hr), so it is likely that the long gut residence times contribute to the high level of assimilation efficiency.

The high water content and low protein content of kangkong, when combined with the much poorer assimilation efficiency shown by river terrapins fed on plant rather than animal material, have implications for the rearing/conservation programmes in Malaysia. Roughly speaking, a river terrapin will have to eat six times as much kangkong as trash fish (on a wet weight basis) to absorb the same amount of energy, and nine times as much kangkong as fish to assimilate the same amount of protein. Given these differences it would appear that reliance on kangkong feeding alone for the youngest of river terrapins is unwise. Moll (1980) reported that growth of hatchlings in the hatchery was much faster than in the wild (a desirable feature in any headstarting operation), but he was reporting data collected at a time when hatchlings were regularly fed fish. Although kangkong is inexpensive, it is probable that weekly feeding on fish (in addition to daily feeding on kangkong) would improve growth rates considerably, thereby reducing the headstarting period. However, more study in this area is clearly desirable.

The finding that river terrapins will not feed at salinities below about 20‰ is consistent with the earlier findings of Davenport & Wong (1986) who found that *Batagur baska* would drink water of 8.7‰ but not 17.5‰. Dunson & Mazzotti (1989) have recently categorized the responses of estuarine/brackish water reptiles. They point out that the simplest adaptation needed to allow a freshwater turtle to cope with saline conditions is for the animal to avoid drinking the external medium when the latter is hypersosmotic to the blood. They also suggest that a reptile which eats numerous relatively small food items, rather than a few large items, must also avoid eating when the external salinity is high because of incidental intake of salty water with the food. This categorization fits *Batagur baska* perfectly. It can and does exploit the productivity of estuaries, but only eats and

drinks when the salinities are below about 15-20‰. River terrapins also tend to exploit bankside vegetation rather than floating vegetation when carried into mangrove channels by the flooding tide (Moll, 1980). Although these behavioural responses appear simple, they do require a degree of neurophysiological adaptation; Dunson & Mazzoti report that freshwater reptiles will usually drink saline water if they are thirsty, a quite inappropriate and ultimately lethal response.

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SHORT NOTES

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 140–142 (1992)

NOTES ON THE DISTRIBUTION AND ECOLOGY OF *PHRYNOCEPHALUS CLARKORUM* ANDERSON & LEVITON 1967 AND *PHRYNOCEPHALUS ORNATUS* BOULENGER 1887 IN AFGHANISTAN

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P. clarkorum and *P. ornatus* are two of the six species of toad-headed agamid lizards that inhabit the desert and semi-desert wastes of southern Afghanistan. The other species are *P. euptilopus* Alcock & Finn 1896, *P. lutteoguttatus* Boulenger 1887, *P. m. maculatus* Anderson 1872 and *P. scutellatus* (Olivier 1807). All these are adapted to the varying ecological conditions which range from large sand seas with massive dune formation, firm sand spits and ridges contiguous with or separate from the sand desert, gravel plains and stony wastes. The abrupt juxtaposition of these ecosystems in some instances allows for a degree of sympatry. Other reptiles are similarly dependant on micro-environmental differences resulting in a rich spectrum of species. The greatest abundance of any given species is often to be found on the margins of its ecological zone.

The two species here considered were originally confused by Boulenger as a composite species: *P. ornatus*, type locality between Nushki and Helmand Afghanistan. Material collected by the present author in 1964 from near Kandahar and Girishk was examined by Steven Anderson and Alan Leviton who recognised that two species were involved and that Boulenger's type series was composed of two distinct taxa. These species were first separated and defined by Anderson & Leviton in 1967 based on the author's 1964 material, specimens collected by John Gasparetti in Afghanistan, animals from Chagai District in West Pakistan and a part of Boulenger's original material amounting to 13 examples of *P. clarkorum* and 24 of *P. ornatus*. Anderson & Leviton (1967) remarked: "whether or not these two similar forms inhabit the same habitats and to what extent their distributions overlap are questions necessitating further investigation". Clark *et al.*(1969) stated: "it would be interesting to know what ecological and behavioural differences may distinguish these two similar and previously confused species". At the time the only field data available from Afghanistan was that made by the present author in 1964. *P. clarkorum* had been taken from near Kandahar and *P. ornatus* from Girishk. In 1968 the author revisited Afghanistan and collected larger samples of both species amounting to 38 *P. clarkorum* and 90 *P. ornatus*. Evaluation of the field data from the trip reveals that these two species can indeed be sympatric and that they demonstrate certain behavioural differences even though they live essentially under the same conditions.

Distribution. Both species have a distribution that is known to include the sandy deserts of southern Afghanistan with some extension into the adjoining regions of West Pakistan and, in the case of *P. ornatus* at least, Iran (Leviton & Anderson, 1970; Welch, 1983). To this region they seem to be endemic along

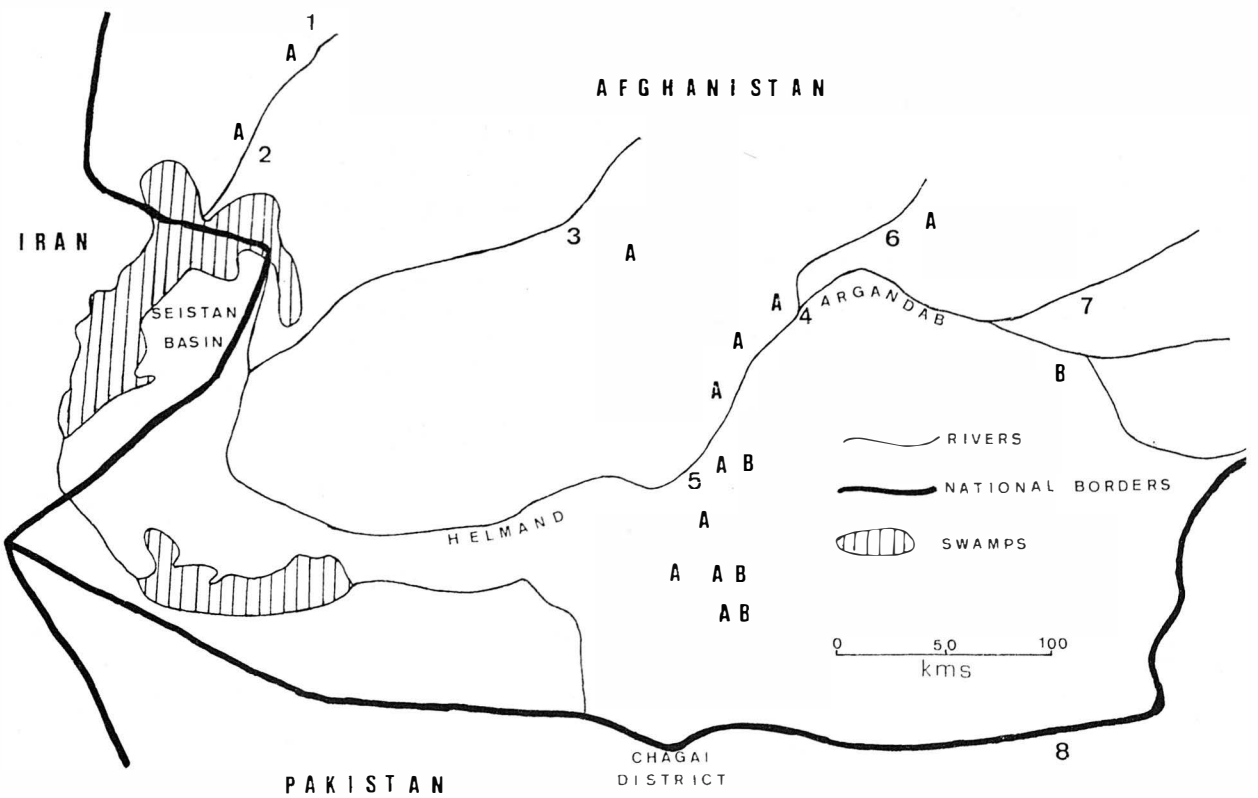

Fig. 1. Map of Afghanistan. 1, Farah; 2, Juwain; 3, Delaram; 4, Lashkargah (Chah-i-Angir, see Leviton, 1959); 5, Darweshan; 6, Girishk; 7, Kandahar, 8, Nushki. A, locality records for *P. ornatus*; B, locality records for *P. clarkorum*.

with the other species referred to above with the exception of *P. m. maculatus* which occurs also in Syria and Iraq (Welch, 1983). Locality records of *P. clarkorum* and *P. ornatus* are few due to the lack of observations in a region that is difficult to research. These localities are shown on Fig. 1. Bearing in mind the known range parameters it is certain that both species occur widely through the area although this remains to be proven. The range of *P. clarkorum* is defined by the Argandab and Helmand river systems which contain the main sand desert in southern Afghanistan. However *P. ornatus* is to be found outside this defined zone, the explanation being that it has followed sand migration patterns which occur as a result of wind action (Clark, 1990). Sizeable though localised sand accumulations from east of Delaram to Kandahar contained populations of *P. ornatus* as well as west of the Helmand river at Lashkargah, Farah and Juwain. At a location 16 km west of Delaram and another 40 km south east of Kandahar none were found. In the former case it could be argued that the site was too far removed from the main sand desert but this does not hold true for the Kandahar station. Altitude may however be significant. *P. ornatus* was not found above 880 m. at Girishk. The altitude at Kandahar was 1100 m. and here *P. clarkorum* occurred. Within the confines of the sand desert itself the two species were sympatric in two areas: 10-20 km north east of Darweshan at 830 m altitude and 55-70 km southeast at 790 m. At the aforementioned Darweshan location *P. clarkorum* was mostly seen in a single broad depression amongst the dunes, the ground being covered with coarse-grained sand. Fewer were observed on the firmer, finer sand on the surrounding sand hills. From the other stations the impression gained was of small well-separated colonies comprised of just a few lizards. Other places visited in the Darweshan area, which was researched extensively over the period 13 March to 4 April, yielded populations of *P. ornatus* but no further *P. clarkorum*. With regard to relative abundance it was the locality near Kandahar that produced most *P. clarkorum*: 21 out of the total of 38 on a single day, 7 April. The remaining 17 examples were caught over a 4 day period. The largest number of *P. ornatus* taken from a single site on a single day was 45 km south of Lashkargah on 13 March when 23 were obtained. This station is isolated from the main desert. On available evidence it seems that whereas *P. ornatus* is more opportunistic and less territorially restricted than *P. clarkorum* both species are more successful where they are not sympatric. Where sympatry occurs both live in reduced numbers and roughly equal population densities. Although no difference in general biotope could be determined, within the limitations already mentioned, the way these species behaved and reacted was at variance. This cannot be adequately discussed without first considering the morphological characters of these two lizards.

Morphology and behaviour. The reader is referred to Anderson & Leviton (1969) for a detailed account of these species and the characters separating them. The most obvious and distinctive features are here given. *P. clarkorum* has a more slender habitus than *P. ornatus* which is rather plumper in body form. *P. clarkorum* has a prominently striped pattern: a broad dorso-lateral and lateral white band bordered with black, the dorso-lateral band extending down the tail. *P. ornatus* lacks these features. *P. clarkorum* has a sandy-grey ground colour with two rows of dark spots down the dorsal aspect; *P. ornatus* has a more brown ground colour with dark spots encircled with orange. In *P. clarkorum* the underside of the tail is lemon-yellow with five transverse black bands; *P. ornatus* has four such bands. In a recent paper (Clark, 1990) it was erroneously stated that both species had up to four black bands. This was a typing error which was overlooked in proof-reading. The black-banding is more striking in *P. clarkorum* not only because there are more bands but due to the fact that they are broader than in *P. ornatus* and that the tail is shorter in proportion to the body length with the interspaces correspondingly reduced. These differences are of great significance in relation to habitat and behaviour. The habitat requirements have already been mentioned: firm sand accumulations or sand-strewn alluvium. In such situations a certain amount of vegetative cover is often present: grassy tussocks, stunted bushes, degraded tree trunks, low xerophytic shrubs. *P. clarkorum* would invariably seek the shelter of any vegetation present when alerted and the striped patterning rendered it inconspicuous when motionless due to the sunshine/shadow factor. Where loose sand was present it would tend to partially bury its hind quarters though never completely covering itself with sand. *P. ornatus* seldom sought such protection preferring to stop in its tracks and remain motionless in the open where its mottled pattern blended with the granular texture of the background sand. It was also noted that *P. clarkorum* would run in shorter dashes and often change direction when pursued whereas *P. ornatus* ran faster and straighter. There was a tendency for *P. ornatus* to seek vegetative cover in places where *P. clarkorum* was not present but never to the same degree of persistence. When under threat both species would raise the tail and lash it from side to side but in flight *P. ornatus* would run with the tail raised whereas *P. clarkorum* never did. This can perhaps be attributed to the fact that in motion a striped or banded pattern creates an illusionary impression which makes it difficult for a potential predator or enemy to follow the movement. Since *P. clarkorum* has a striped pattern it does not need to raise the tail to delude the pursuer. *P. ornatus*, lacking body striping, raises the tail to create the same effect and when the animal suddenly halts in its tracks the tail is lowered rendering the animal invisible at a distance from its pursuer that is hard to determine. The two species under discussion are not alone in 'tail display' which is a characteristic of the genus as a whole and a feature of all Afghan species. This has been discussed by the author recently (Clark, 1990). It can further be construed as a warning/threat gesture as well as a recognition signal during courtship. Nikol'skii (1911) gives a full and vivid description of tail display in the Central Asian *P. sogdianus* (=*P. interscapularius*). This species is also found in northern Afghanistan and closely parallels both *P. clarkorum* and *P. ornatus* though would seem to have closer affinities to the latter. He concludes that "the bending of the tail is important only as a countermimic adaptation". Clearly more research is needed in this field and the reasons why different species adopt, dissimilar display patterns.

Reproduction. With regard to the reproductive cycles of *P. clarkorum* and *P. ornatus* we have little data. Anderson & Leviton (1967) state that ovarian eggs were present in both species in animals examined by them which had been collected between May and October. Two *P. clarkorum*, taken on May 15th. 1962 in Chagai District in Baluchistan had oviducal eggs implying that egg deposition had not yet begun or that it was at any rate not completed. Of the present author's material collected early in April a greater number of *P. clarkorum* contained oviducal eggs than did *P. ornatus*, in proportion to the total collected. This suggests that the breeding season is perhaps staggered by a month or so with *P. ornatus* completing egg deposition by around the middle of April and *P. clarkorum* a month or so later. It was also found that *P. ornatus* was active at lower environmental temperature than *P. clarkorum*, the former being found on 26 February near Girishk around midday with air and ground temperatures of 15.5°C and 26°C respectively. *P. clarkorum* was never found with an air temperature lower than 24°C. This suggests that *P. clarkorum* is more sensitive to low temperatures with a longer period of hibernation which

would result in it breeding later. Clearly, these conclusions are based on limited information and await confirmation by more detailed studies.

Conclusions: *P. clarkorum* and *P. ornatus* are sand-dependent lizards with preferred habitats of firm sand and sand-strewn alluvium. *P. clarkorum* is intimately connected to the main desert sand formations, *P. ornatus* ranges more widely following migratory sand accumulations. Where sympatric both occur in stable and compatible populations neither showing dominance but are less abundant than in areas where either one or the other species is found. Behaviour, escape methods and detection avoidance can be related to morphological differences. *P. clarkorum* seems to breed later than *P. ornatus* and is probably more sensitive to lower temperatures.

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VARIATION IN VIABILITY DURING DEVELOPMENT AND HATCHING SUCCESS IN EMBRYOS OF THE TOAD *BUFO CALAMITA*

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Many authors have dealt with embryo hatching success in anurans as failures in fertilization efficiency (Davies & Halliday, 1977; Gerhardt *et al.*, 1987; Ryan, 1985; Robertson, 1990). However, other sources may contribute to additional embryo mortality throughout development: these include failures or abnormalities in development, fungal infestations prior to hatching, etc. (Herreid & Kinney, 1966; Woodruff, 1976; Seigel, 1983; Travis *et al.*, 1987; Banks & Beebee, 1988). Therefore, any attempt to separate fertilization efficiency from later factors governing mortality is an important matter that ultimately relates to sexual selection and fitness, since Licht (1976) proposed that an optimal male/female body size ratio might enhance fertilization rate.

Little information is available regarding embryonic development and viability of the next generation, and non-proximate factors such as female body size and age. Other factors such as egg size may affect some components of offspring fitness such as embryo size at hatching (Kaplan, 1980; Crump, 1984). However, no information is available concerning embryo survival and egg size. Finally, external fertilization in anurans may result in a lower rate of successful insemination at higher clutch sizes.

The present study examines the variation in embryo viability during development from fertilization to hatching in the natterjack toad, *Bufo calamita*. Fertilization success is analyzed as a function of body size ratio (defined as relative male body length/female body length). Likewise, both fertilization efficiency and hatching success are studied as a function of female body size, age, and clutch and egg size.

The study was conducted in central Sierra Morena, Córdoba Province, Spain, from January to April of 1987 and 1988. Naturally-occurring pairs of natterjack toads in amplexus were captured in the breeding area; 71 in 1987 and 44 in 1988. The pairs were isolated in glass aquaria (30 x 20 x 20 cm) filled with 3 l of pond water and some vegetation. The aquaria were placed at the site where the pair had been captured until oviposition occurred. Then the individuals were measured for body length and toe-clipped. Toes were frozen for skeletochronological study in order to determine successive resting lines, providing an estimate of the age of each individual (Tejedo, 1989). All clutches were photographed to estimate clutch size and were then carefully released into the pond. The absolute number of eggs for each clutch was counted from the resulting photographs. Average egg size was determined by measuring the diameter of ten eggs from samples randomly selected in each clutch, to the nearest 0.02 mm, using an ocular micrometer. Estimates of embryo viability were obtained from samples taken sequentially from individual clutches. Embryos were staged according to Gosner (1960). In 1987, four samples were taken at 3-4 day intervals: (1) late cleavage, stages 9-10, taken about 12 hr after oviposition. This sample was used as an estimate of fertilization efficiency; (2) late gastrula or early neurula, stages 12-13; (3) neurulation, stages 14-16; and, (4) hatching, stages 17-19. During 1988, only two samples were taken, spaced about 10 days apart: (1) stages 9-10, taken about 12 hr after oviposition (fertilization success estimate); and (2) stages, 16-18. The final samples from both years were used to estimate total number of viable embryos and percent of hatching success. Additionally, collections were taken from non-manipulated clutches, those laid freely, not in aquaria ($N = 10$ in 1987 and $N = 6$ in 1988), to ascertain if manipulation affected total number of viable embryos. Manipulated clutches did not differ from control ones in clutch size or average egg size (Mann-Whitney U-test, $P > 0.05$). The average sample size ($\bar{x} \pm$ SD) was 71.2 ± 33.2 embryos, $n = 246$, in 1987, and 85.4 ± 37.4 embryos, $n = 100$, in 1988. Each sample was immediately examined with a dissecting microscope. Embryos were counted and scored as non-viable or dead when they were grey and swollen. Dead

SAMPLE	1	2	3	4
1		3.732 **	4.307 ***	3.361 *
2			0.348 NS	0.181 NS
3				0.521 NS

TABLE 1. Non-parametric Tukey multiple comparisons analysis (q) of percent of viable embryos of successive samples taken from clutches during the 1987 breeding season. $\bar{x}$ ± SD of survival rate in each sample were: 1st sample, 95.18 ± 10.38%, N = 71; 2nd sample, 84.30 ± 18.14%, N = 41; 3rd sample, 81.05 ± 20.22%, N = 43; 4th sample, 80.44 ± 27.59, N = 71.* $P < 0.005$; ** $P < 0.002$; *** $P < 0.001$

embryos were quickly covered by fungi, hence estimates of embryo survival in the later samples were made by examining the proportion of embryos either infected by fungus hyphae or decomposed.

A comparison of percent of embryo surviving during 1987 revealed significant differences between succesive samples (Kruskal-Wallis test, H = 24.34, df = 3, $P < 0.0001$, Fig. 1). *A posteriori* non-parametric Tukey multiple comparisons analysis revealed particular differences (Table 1). Embryos in Gosner stages 9-10 had a significantly higher mean survival than subsequent samples. These subsequent samples showed no significant differences when compared to each other (Table 1). Comparison between the two samples taken in 1988, however, failed to reveal any significant difference (1st sample $\bar{x}$=94.72%, SD=14.9, N=44; 2nd sample $\bar{x}$=93.71%, SD=13.6, N=44, Mann-Whitney U-test, Z=1.22, P=0.11). Some authors have found that most embryo mortality in amphibians occurs during gastrulation and neurulation (Anderson *et al.*, 1971; Smith, 1974 in Cooke, 1975; Woodruff, 1976, Travis *et al.*, 1987). A possible explanation of the differential mortality recorded during this period may be that gastrulation is a critical process in development which involves important interaction between the nuclear genome and cytoplasmic proteins initiating differentiation in embryos (Sargent & Dawid, 1983). This process may give rise to strong sensitivity in embryos at this stage. All dead embryos were quickly covered by fungi but these may have been saprophytic. Some authors have observed fungal contamination of dead amphibian embryos (Herreid & Kinney, 1966; Tilley, 1972; Cooke, 1975; Woodruff, 1976; Banks & Beebee, 1986, 1988) but only Villa (1979) could find clear evidence of the parasitic role of fungi on developing embryos.

Fertilization success was similar in both years of study (1987, $\bar{x}$=95.2±10.4 %, N=71; 1988, $\bar{x}$=94.7±14.9, N=44, Mann-Whitney U-test, Z=0.85, P>0.20). Manipulated clutches did not differ from control clutches in average fertilization success (Mann-Whitney U-test, 1987, Z=0.98, P>0.10; 1988, Z=0.32, P>0.20). This suggests that the confinement of eggs and toads to an aquarium did not result in an unnaturally high fertilization success. Male/female body length ratio was not correlated with fertilization success either in 1987 (r_s=0.084, P>0.20, N = 71) or in 1988 (r_s=0.045, P>0.20, N=44). This result does not support Licht's (1976) prediction of an optimal male/female size ratio which would enhance fertilization efficiency. Some authors have verified this prediction (Davies & Halliday, 1977; Ryan, 1985; Gibbons & McCarthy, 1986; Robertson, 1990); however others have shown that no such relationship exists (Kruse, 1981; Gerhardt *et al.*, 1987; Höglund & Robertson, 1987; Krupa, 1988). The differences observed among these species seem to be unclear.

Female body size ranged between 55.5 and 93.5 mm, with an average value of 72.95±8.49 mm, N=115. Female size was not related to fertilization success (1987, r_s=0.034, P>0.20, N=71; 1988, r_s=0.123, P>0.20, N=44). Age was estimated only in 1987 from a sample of N=41 mated females. Averaged age value was 4.49±2.3 resting lines, range 2-10. Female age variation was also independent of fertilization success (r_s=0.17, P>0.20, N=41). The average egg size ranged between 1.37 and 1.93 mm, $\bar{x}$=1.69±0.11 mm, N=95 clutches. Egg diameter was also unrelated to fertilization success (1987, r_s=0.084, P>0.20, N=51; 1988, r_s=-0.08, P> 0.20, N=44). The average number of eggs per clutch was 3818.9±1373.5 eggs, (range 1234-8840, N=115). Likewise, clutch size was unrelated to fertilization success (1987, r_s=0.084, P>0.20, N=71; 1988, r_s=0.11, P>0.20, N=44). The average hatching success was significantly lower in 1987 ($\bar{x}$=80.4±27.6 %, N=71 clutches) than in 1988 ($\bar{x}$=94.3±8.97, N=44 clutches; Mann-Whitney U-test, Z=2.92, P=0.002). Environmental factors may induce the observed variability. However, it is not possible to account for the differences in hatching success observed between years. Manipulated clutches did not differ from undisturbed control clutches in mean hatching success during either year (Mann-Whitney U-test, 1987, Z=0.28, P>0.20; 1988, Z=0.002, P>0.20). Embryo hatching success was unrelated with female body size (1987, r_s=0.097, P> 0.20, N=71; 1988, r_s=-0.129, P>0.20, N=44). Variation in female age was also independent of hatching success (r_s=0.146, P>0.20, N=41). Mean egg size and clutch size was not correlated with hatching success (egg size, 1987, r_s=0.167, P>0.20, N=51; 1988, r_s=0.025, P>0.20, N=44. Clutch size, 1987, r_s=-0.126, P>0.20, N=71; 1988, r_s=0.130, P>0.20, N=44).

Fertilization success recorded in this study was similar to that found in other studies in which average values ranged from 78-96%, with the exception of notably lower values found in *Bufo calamita* by Banks & Beebee (1986). The difference may lie in the fact that the senility effect, responsible for lower efficiencies in older individuals from the British population, was not observed in this study. Female body size or age influenced neither embryo survival at early stages nor hatching success. Moreover, reproductive traits, such as egg size, did not affect embryo survival. This is not consistent with models which predict a correlation between egg size and offspring fitness (e.g. Smith & Fretwell, 1974). Larger clutches did not present a lower fertilization success, hence sperm cost does not seem to limit fertilization potential as has been suggested in other species (Robertson, 1990). Variation in embryo survival and hatching success in *Bufo calamita* may be mediated by random mechanisms or perhaps by environmental factors not measured in this work.

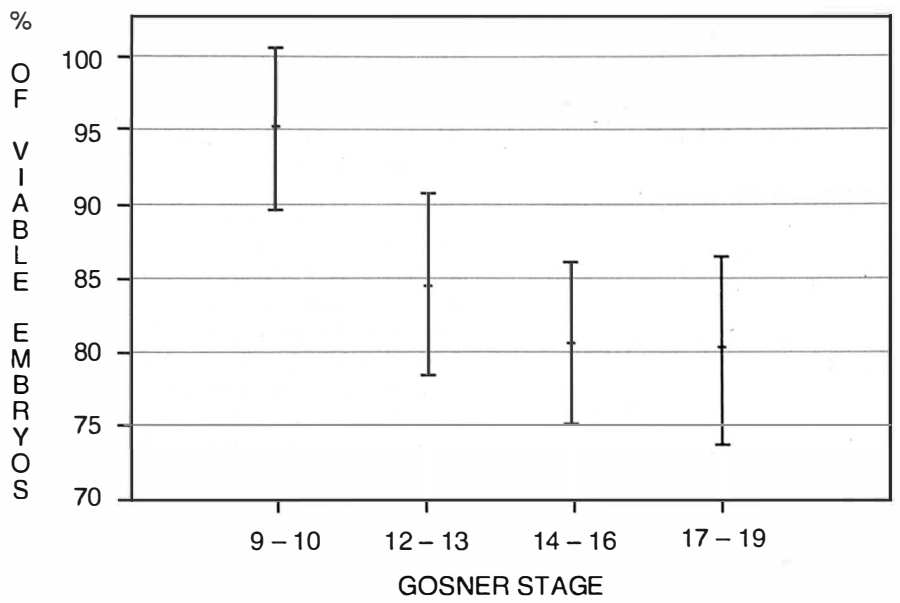

Fig. 1. Percent of embryos alive in four successive samples taken from clutches of *Bufo calamita* during the 1987 breeding season. Embryos from each sample were in the Gosner stages indicated. Mean values are indicated and bars represent 95% confidence intervals.

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SNAKE LITTER SIZE = LIVE YOUNG + DEAD YOUNG + YOLKS

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A common method of estimating litter size of viviparous snakes is to hold gravid females in captivity until they give birth. Captive-born litters often include dead young (either partly or fully developed), or undeveloped yolky eggs, or both. Because we do not know the extent to which captive conditions influence the occurrence of dead young or undeveloped eggs, it is not clear what constitutes a reasonable estimate of litter size: live young only or live young plus some subset of the remaining offspring and eggs. Different authors have used slightly different criteria (e.g. Gregory, 1977; Larsen, 1986; Ford & Seigel, 1989; see review by Farr & Gregory, 1991), but undeveloped eggs seem to be rarely, if ever, counted as part of the litter.

In this note, we examine the degree to which estimates of litter size are influenced by the inclusion or exclusion of dead or undeveloped young, using data for the garter snake, *Thamnophis sirtalis*, from ten Canadian populations.

Gravid females were captured at various stages of gestation in various years from 1972 to 1988 and maintained in more or less standard conditions at 20 - 28°C, with *ad lib* access to water but usually no food, until they gave birth, whereupon measurements of litters and neonates were taken.

We classified each litter into one of three categories, following Farr & Gregory (1991):

Status 1 – litters that consisted only of live young.

Status 2 – litters that consisted entirely of fully developed young, of which some or all were dead.

Status 3 – litters that contained one or more dead, incompletely developed young and/or undeveloped eggs.

In order to compare estimates of litter size among categories, we first had to consider that there were significant differences among locations in frequencies of the three litter categories and in the linear relationship between litter size and snout-vent length (SVL) of mother. Therefore, we calculated a separate regression of litter size on SVL of mother for each location, using Status 1 litters only. We then expressed sizes of Status 2 and 3 litters from each location as deviates from the corresponding Status 1 regression line. We did this three times, using (i) live young, (ii) live plus normal dead young, and (iii) all "progeny" as measures of litter size. Because the variances around the various regression lines were all different, we re-expressed each value as a standard normal deviate by dividing it by the standard deviation of the residuals from the appropriate regression line. We then pooled the data from all locations and compared mean adjusted sizes of Status 1, 2, and 3 litters by ANOVA.

We also tested whether neonate SVL differed among litter categories. First, we compared the SVLs of live and dead babies in Status 2 and 3 litters by two-way ANOVA (individual litter by live versus dead young), using the General Linear Model. Second, we compared SVL of neonates (using both live and dead young) among litter categories, again as a two-way ANOVA (location by litter status) of mean SVL of neonates in a litter. Location was used as a factor in the ANOVA because neonate SVL differs significantly among locations (Gregory & Larsen, unpubl.).

To test for potential influences of captivity on incidence of Status 2 and 3 litters, we did a one-way ANOVA of days in captivity (all locations combined) with litter status as the factor.

All statistical analyses were done with PC-SAS Release 6.03 and conclusions were based on Type III sums of squares. All were considered significant at alpha = 0.05.

Of 162 captive-born litters, 83 were Status 1, 41 Status 2, 38 Status 3, but not all could be used in all analyses. Mean litter size differed significantly among the three categories, regardless of definition of litter size, but these differences became progressively smaller and less clear-cut as the definition of litter size was broadened (Table 1). Although Status 2 and 3 litters were smaller than Status 1 litters when only live young were considered, both turned out to be larger than Status 1 litters when all potential components of litters were summed.

	Litter Size		
	Live Young	Live Young + Fully developed Dead Young	All "Progeny", Including Undeveloped Young and Yolks
Status 1 (n=72)	0.000	0.000^{A}	0.000^{A}
Status 2 (n=27)	-3.300^{A}	1.469	1.469^{B}
Status 3 (n=31)	-2.338^{A}	-0.510^{A}	0.747^{AB}
ANOVA (df = 2,127)	F = 9.20 P = 0.000	F = 5.24 P = 0.006	F = 3.55 P = 0.031

TABLE 1. Comparison of mean litter sizes of Status 1, 2, and 3 litters under different definitions of litter size. All values were calculated as standardized normal deviates from separate regression lines of litter size vs. female SVL for Status 1 litters of each population. Expressing values as deviates adjusts for differences in SVL of mothers. Standardizing values against a normal distribution eliminates heterogeneity of variance among populations, allowing them all to be pooled for final analysis. By definition, mean deviation of Status 1 litters is always zero. Symbols A and B indicate means with non-significant difference (Bonferroni test).

We compared SVLs of dead and live young within litters, using nine litters for which we had at least two measurements of each of live and dead young (most litters selected had more than two of each). There were significant differences in mean SVL among litters ($F_{8,118} = 113.37$, $P = 0.0001$), but not between live and dead neonates within litters ($F_{1,118} = 1.07$, $P = 0.0731$); the interaction factor was not significant ($F_{8,118} = 0.920$, $P = 0.545$).

We restricted our comparison of SVLs of neonates from litters of different status to three locations for which there were at least three litters of each status. Neonate size differed significantly among these locations ($F_{2,39} = 15.06$, $P = 0.0001$), but not among status categories ($F_{2,39} = 1.58$, $P = 0.222$); the interaction was not significant ($F_{4,39} = 1.11$, $P = 0.3701$).

There were no significant differences in mean time spent in captivity by females producing litters of different status ($F_{2,112} = 0.79$, $P = 0.4551$).

Perforce, inclusion or exclusion of dead young or undeveloped eggs must change estimates of litter size. However, the important conclusions of this study are that this effect can be very significant in snakes and that variation among estimated litter sizes is maximally reduced by counting all potential progeny. This supports the recommendation of Farr & Gregory (1991) that a distinction be made between potential and actual litter size. At the very least, all potential components of litters should be reported in future studies, regardless of which of them are considered to be important. A case in point is that of Ford & Karges (1987), who obtained higher estimates of litter size in *Thamnophis marcianus* from live births than from counts of embryos. The factors that reduce the apparent potential size of some litters merit further study.

Preferably, estimates of actual litter size should include all normally developed dead young. We have no evidence that dead young are smaller or differ in any other obvious way from live young, in general. Stillbirths and deformed young occur in the wild (Larsen, 1986), but their incidence may be higher under captive conditions. A number of factors could have a negative influence, including death, on developing progeny in captivity (see Farr & Gregory, 1991, for review); chief among them is temperature (Fox, 1948; Burger *et al.*, 1987; Burger & Zappalorti, 1988; Burger, 1990), which we did not attempt to optimize in this study. Why some embryos in a litter die while others survive is not known. It is interesting to note that the adjusted sizes of Status 2 litters were significantly larger than those of Status 1 litters when both live and dead young were counted (Table 1). Perhaps there is a crowding effect *in utero* that does not manifest itself until late gestation, resulting in fully developed, but dead, young. Farr & Gregory (1991), however, found no significant differences in sizes of litters of *T. elegans* of different status when all potential litter components were counted.

It is not clear whether the undeveloped eggs seen in Status 3 litters were simply unfertilized vitellogenic follicles or fertilized eggs that ceased development at a very early stage. Clearly, some embryos do cease development in mid-gestation because some Status 3 litters were so classified on the basis of undeveloped young. Again, we have no field data on the occurrence of undeveloped eggs or young in litters. Whatever their significance, their inclusion as a component of potential litter size is important because such inclusion allows unambiguous comparison between different populations and different studies. This is especially true if we want to compare data obtained from captive births with those from hand palpations in the field (cf. Farr & Gregory, 1991) or from dissections of females in early stages of pregnancy; in neither of these cases will it usually be possible to distinguish eventual live births, dead births, or undeveloped eggs.

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BOOK REVIEWS

The Year of the Turtle. D. M. Carroll. (1991). 172 pp. Camden House Publishing, Charlotte, Vermont. $22.95(US), cloth. $17.95(US), paper.

It is popular among advertising agencies and writers of second-rate fiction to picture scientists as detached, emotionless beings peering over the edge of a clip board at "the phenomenon", clad in a white lab coat and thick glasses, armed with reams of stop-watches, pens and calculators. Yet what fool would endure years of university education, spend countless hours reading terse, factual articles, and then submit himself to

the rigours of lonely months or years of field or laboratory work if at heart he did not feel a passionate love for the object of this torture we call science?

It is difficult to show love without slipping into sentimentality, but this is exactly what David Carroll achieves with *The Year of the Turtle*. For a natural historian or field biologist there is no greater expression of the beauty and mystery of nature than simple, direct, elegant description. Carroll's book follows a year in the life of a small marsh, from spring and the excitement of finding animals as they emerge from hibernation through to the close of autumn and dormancy for the turtles.

This is neither *Tales of the River Bank* nor *Teenage Mutant Ninja Turtles*, but a set of chronicles drawn from years of intimate observation. Written in a frank, straight-forward style which evokes with perfect clarity the colour and mood of the marsh, and admirably illustrated by the author, this book is the perfect introduction to the natural history of a North American marsh. Although there are no graphs or statistical analyses here, this book is of great value to scientists interested in the life-history of freshwater turtles in a way that is not fashionable: through careful observations and accurate descriptions, one is left with a feeling for the annual rhythm of the marsh and its inhabitants which is lost when observations are distilled into completely objective statistics. Wondrously, this feeling is available to everyone, regardless of specialist training. Great advances in the study of life-history evolution of turtles are now being made: witness the recent publication of *Life History and Ecology of the Slider Turtle*, edited by J. Whitfield Gibbons (Smithsonian Press, Washington). We need to continue to persue our understanding of the ecology of turtles in terms of fitness and clutch size and annual energy budget and other objective measures. We also need to recognize and enjoy the intangible love of nature which keeps us at it. This is a highly readable book, and is the perfect companion to sorting out last summer's field notes on those long winter nights.

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Serpientes de Ecuador. C. Perez-Santos & Ana G. Moreno. (1991). 538 pp.Museo Regionale di Scienze Naturali, Torino (Monografia XI). Spanish.

This is the second monograph by Perez-Santos and Moreno to address the ophidian fauna of a specific South American country and it follows the same format of their previous contribution, *Ofidios de Colombia* (1988; Monografia VI). Although written in Spanish this book is quite easy to follow as each of the species accounts, which make up the bulk of the tome, follows the same format with taxonomic and distributional data laid out in tabular form and the reader is soon able to dispense with the Spanish-English dictionary even for the more descriptive sections. All the dichotomous keys are conveniently published both in Spanish and English and line drawings of the head scutes and body scalation are provided for many species. The forepart of the book contains much useful geographical, ecological and climatological information, including several maps, and a checklist of Ecuadorian snakes. The final part of the text includes a useful section on the snakes of Galapagos - politically part of Ecuador but frequently overlooked in publications on Latin America (Peters, Orejas-Miranda & Donoso-Barras, 1986) - and several tabulated summaries of the origins and distribution patterns of Ecuadorian serpents, followed by 150 full-colour photographs of habitats and species and an extensive bibliography. This book should certainly find a place on the bookshelf of any herpetologist with an interest in the ophiofauna of Latin America.

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Peters, J. A., Orejas-Miranda, B. & Donoso-Barros, R. (1986). *Catalogue of the Neotropical Squamata Parts I & II* (with new material by P. E. Vanzolini). Washington & London: Smithsonian Inst. Press. (revised edition).

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Die Schlangen Europas und rund ums Mittelmeer. Ulrich Gruber. (1989). 248 pp. Franck'sche Verlagsbuchhandlung, W. Keller & Co., Stuttgart. DM 49.80, cloth. (in German).

This is the first field guide covering all European snake species (including the European part of Russia) plus most species occurring in Turkey, Cyprus, the Levantine and North African countries bordering the Mediterranean. This geographical scope makes the book highly useful, especially for travelling herpetologists.

After some introductory remarks, a number of very short chapters treat aspects of snake anatomy, mobility, sensory systems, diet, skin shedding, reproduction, life expectancy, behaviour, venom and venom apparatus, first aid, habitat, enemies, protection, care, and systematics. A key to the genera leads into the descriptive part, which provides for every species information on distinctive characters, habitat, habits, food, reproduction and distribution. The goal of supplying at least one good quality photograph for every species has nearly been achieved: only a few rare species are represented by line drawings. Some species, however, were given a surplus number of pictures; e.g. *Natrix natrix* is shown in five photos, and *Vipera ammodytes* even in twelve, depicting all geographical varieties.

The taxonomic state on which this book is based is of the late eighties. Recently described taxa lacking in older publications such as *Coluber cypriensis*, *Eirenis barani*, *Natrix megalocephala*, *Vipera barani*, *V. wagneri*, *V. dinniki*, *V. bulgardaghica*, *V. lebetina mediterranea*, are included, but *Vipera nikolskii* is missing. *Telescopus tripolitanus* is only briefly mentioned (as a probable subspecies of *T. dhara*). The taxonomy of *Echis* is not up to date; North African specimens are included in *E. carinatus* not in *E. leucogaster* and *E. pyramidum*, as by recent authors.

Desert forms, such as *Cerastes* and *Lytorhynchus*, are covered, but tropical relicts, such as *Atractaspis engaddensis* in Israel and Sinai, *Bitis arietans*, *Lamprophis fuloginosus* and *Dasypeltis scabra* in Morocco, are lacking.

There are a number of shortcomings regarding geographical distribution: no maps are presented, and range descriptions are not very accurate. *Psammophis sibilans* is wrongly noted for the "whole of North Africa"; *Vipera lebetina* is recorded for Israel, where it no longer exists. On the other hand, *Lytorhynchus* is not indicated for Morocco, *Pseudocerastes* not for Sinai, *Vipera aspis* not for Spain (however, the latter is listed in the country checklist at the end of the book, which is one of its merits).

The text is generally accurate, although some statements may be criticized (e.g. that antivenin treatment is regarded as necessary for bites by *Cerastes vipera*).

Altogether, this is a high quality field guide, which I can recommend to any herpetologist, German-speaking or not.

Ulrich Joger
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BOOKS RECEIVED

The Balance of Nature? Stuart L. Pimm. (1991). 434 pp. The University of Chicago Press, Chicago and London. £21.50 paper; £49.50 cloth.

Subtitled *Ecological Issues in the Conservation of Species and Communities*, this book addresses the concept of stability in ecological communities, discusses how it may be applied to conservation biology, and urges a new alliance between theoretical and empirical studies.

Water Economy in the Life of a Terrestrial Anuran, the Toad Bufo bufo. C. Barker Jorgensen. (1991). 30 pp. Biologiske Skrifter 39, The Royal Danish Academy of Sciences and Letters, Copenhagen. DKK 70,00 paper.

This scientific paper describes an extensive physiological study of the water balance of a single species.

Foundations of Ecology. Classic Papers with Commentaries. Leslie A. Real and James H. Brown (eds.). (1991). 904 pp. The University of Chicago Press, Chicago and London. £21.95 paper, £55.95 cloth.

This volume assembles for the first time forty classic papers which have laid the foundation of modern ecology. The combination of classic papers and fresh commentaries makes the book a convenient reference to papers often cited today.

INSTRUCTIONS TO AUTHORS

(revised January 1992)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: full papers (no length limit); reviews and mini-reviews (generally solicited by a member of the editorial board); short notes; controversies, under 'Forum' (details available from the Editor); and book reviews. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance.

2. *Three* copies of all submissions, and illustrations, should be sent to the Editor. All papers will be subject to peer review by at least two referees

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. Typesetting is greatly assisted if accepted manuscripts can be supplied on microcomputer diskettes. Authors are therefore strongly encouraged to produce manuscripts using a wordprocessor (preferably on a PC-compatible microcomputer).

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of 5 words or less. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. Short notes (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. Table I; they should be typed double spaced on separate sheets with a title/short explanatory paragraph underneath.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. Fig. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated ..."; "...as observed by Smith & Jones (1963)." "...as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. The following examples will serve to illustrate the style and presentation used by the Journal.

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

   Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

   Dunson, W. A. (1969a). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

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

9. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

10. Proofs should be returned to the Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work.

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