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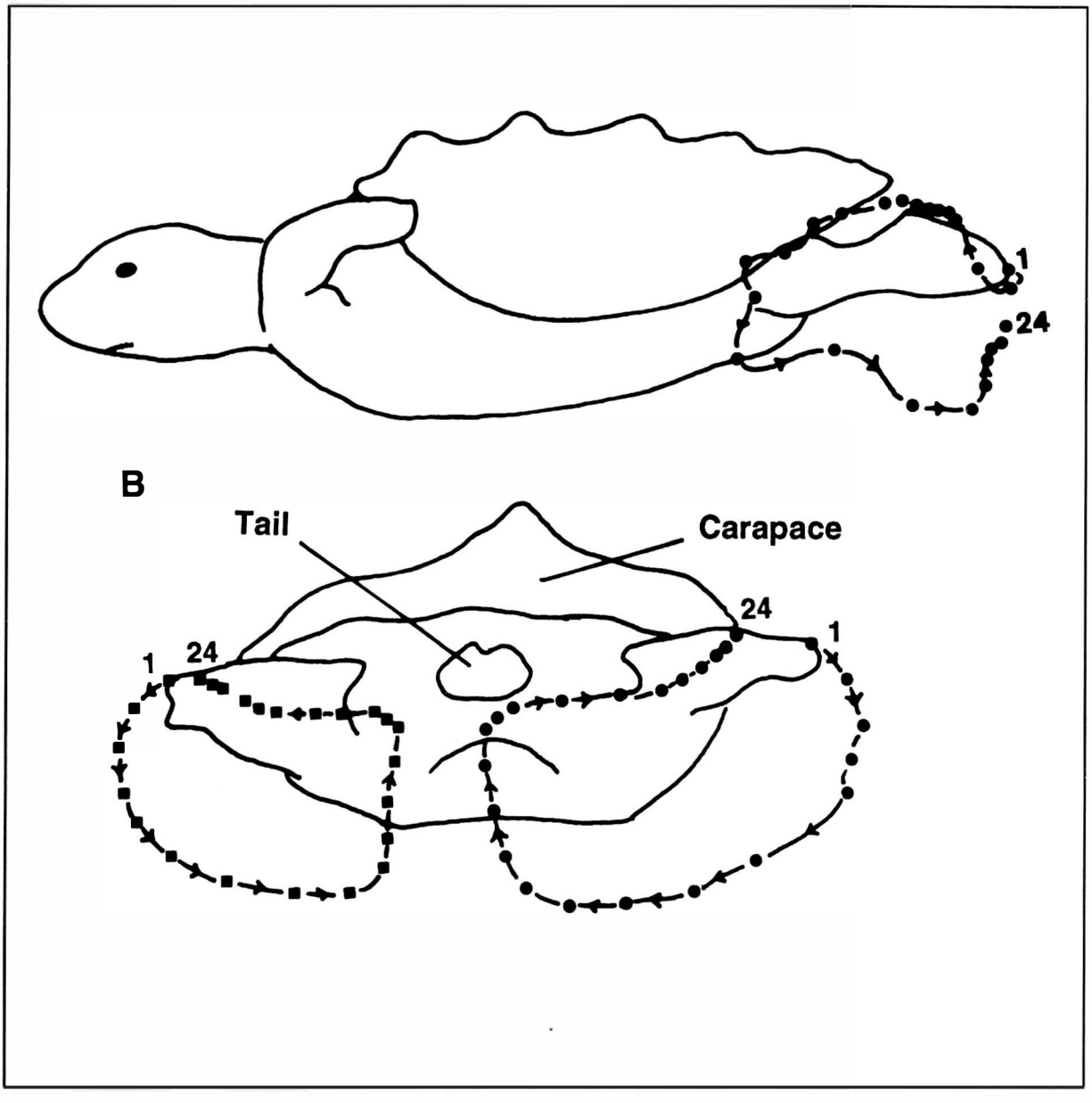

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FRONT COVER: Swimming movements in the Pacific ridley turtle (J. Davenport)

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DEVELOPMENTAL ARREST IN *LEPTODACTYLUS FUSCUS* TADPOLES (ANURA: LEPTODACTYLIDAE). I: DESCRIPTIVE ANALYSIS

J. R. DOWNIE

Department of Zoology, University of Glasgow, Glasgow G12 8QQ, UK

Eggs of the neotropical frog *Leptodactylus fuscus* (Anura: Leptodactylidae) are laid in foamy masses in burrows close to sites of temporary pools. After about two days, tadpoles hatch into the foam, but after two days further development, if no rain has fallen, the tadpoles enter a period of developmental arrest, maintained until rain falls and the nest is inundated. Results reported here document the developmental status of the arrested tadpoles, and compare this with tadpoles that enter water immediately. Arrest involves a gradual slowing and eventual cessation of cell proliferation, a slowing of hatching gland degeneration, a cessation of limb bud morphogenesis and slowing of yolk utilization from intestinal lining cells. Arrest is not simply the result of lack of food, since the intestinal lining contains abundant yolk particles when it begins. The period of developmental arrest is not unlimited: around 20 days after egg deposition, the resultant tadpoles undergo progressive weight loss and eventually die; death occurred on average 27.5 days after egg deposition,under laboratory conditions.

INTRODUCTION

Frogs of the *Leptodactylus* 'fuscus' group (Heyer, 1978) lay in foam nests in burrows on land, near sites of temporary pools, but often in advance of heavy rains (Kenny, 1969; Martins, 1988). In species investigated so far, eggs develop past hatching but, in the absence of heavy rain, the tadpoles enter a form of developmental arrest (*L. prognathus* [= *L. latinasus*; Heyer, 1978] and *L. bufonius* - Pisano & Del Rio, 1968; *L. fuscus* - Downie, 1984; Solano, 1987; *L. mystaceus* - Caldwell & Lopez, 1989) and this may be a general characteristic of the group. In addition, Downie (1984, 1989) showed that in *L. fuscus*, once the post-hatching tadpoles have progressed in the nest beyond Gosner (1960) stage 25, they begin to make a new kind of foam which eventually replaces the collapsing foam produced by the mating parents. This observation has been confirmed in *L. mystaceus* by Caldwell & Lopez (1989). Downie (1989) showed that the new foam is largely made of bubbles which the tadpoles spit out, and presumably contains mucus-rich oral secretions.

Soon after the tadpoles start making foam, further development slows down or stops, and the tadpoles may then remain in a state of developmental arrest for several weeks, awaiting the onset of heavy rains to continue their development.

Pisano & Del Rio (1968) contended that *L. prognathus* (*latinasus*) and *L. bufonius* tadpoles in foam nests showed inhibition of growth, but that morphogenesis and differentiation continued normally. Unfortunately, they were unaware of the foam-making activities of the tadpoles, nor did they determine the developmental stage at which inhibition occurs, and it is therefore difficult to judge their conclusions.

The principal aims of this paper are to document what happens during developmental arrest in *L. fuscus*, and to describe changes in the tadpoles that occur if the arrest period is prolonged.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF TADPOLES

Foam nests of *L. fuscus* were collected from burrows around the margin of a temporary pool site on the University of West Indies campus at St Augustine, Trinidad, and from the banks of drainage ditches in St Augustine and near Piarco Airport during June- August 1982, 1987, 1989 and 1991. On collection, a few eggs or tadpoles were removed for staging, using Gosner's (1960) normal table, and generally fixed for storage in Bouin's fluid. Foam nests were maintained in the laboratory on the surface of moist paper tissue in polythene tubs. Daily laboratory temperature fluctuation was 25-28°C. The results reported here are all based on nests collected at stages before or just after hatching: once tadpoles have started making foam, it is difficult to be sure when the eggs were laid, and this interferes with the documentation of changes during the arrest period.

Tadpoles were removed at intervals and fixed in Bouin's fluid for measurement, staging and histological processing. To compare developmentally arrested *L. fuscus* tadpoles with tadpole growth once food was available, some foam-making tadpoles were transferred to 2 litre polythene tubs containing 1.5 litres dechlorinated tap water and fed with powdered aquarium fish food. Samples were fixed after 1, 2 and 3 days growth.

For an out-group comparison, floating foam nests of another Trinidad Leptodactylid, *Physalaemus pustulosus* were collected in similar localities to *L. fuscus*. Tadpoles of this species do not remain long in the nest after hatching (Downie, 1992): tadpoles were maintained in glass tanks in aerated dechlorinated tap water, fed on powdered aquarium fish food, and fixed in Bouin's fluid as required.

For general accounts of the species used, see Kenny (1969) and, for nomenclature changes, Harding (1983).

MEASUREMENT AND STAGING

The length of each fixed tadpole was measured using a Wild M5 binocular microscope with calibrated eyepiece graticule at x6 objective magnification. Two length measurements were generally taken: total length and body length, defined as the distance from snout to base of hind limbs.

Hind limb buds were drawn and measured using a Wild Drawing Tube at x25 objective magnification to assess developmental stage (Gosner, 1960).

For wet weights, fixed tadpoles were damp dried with tissue, then weighed to the nearest 0.1 mg using a Sartorius research balance. After taking wet weights, samples of fixed tadpoles were dried and re-weighed to the nearest 0.1 mg.

Whole tadpoles were photographed using a Wild M3Z stereomicroscope, Wild camera and Ilford Pan F film.

HISTOLOGICAL PROCESSING AND EXAMINATION

After measurement and staging, samples of tadpoles were embedded in paraffin wax and serially sectioned transversely at 7 μm. Anterior sections (from the snout to just behind the eyes) were stained with Periodic acid Schiffs (PAS) and light green; posterior sections were stained with Haemalum and Eosin. Sections were examined with a Wild M20 microscope (with the aid of a Wild Drawing Tube when drawings were required). Photographs were taken using a Leitz Dialux microscope with Wild camera and Ilford Pan F film.

QUANTITATIVE MEASUREMENTS

The following measurements were made:

(a) Hatching gland cell (HGC) numbers at different stages were assessed by noting whether or not they were present at the extreme anterior end of a tadpole, and by counting them at two locations: these were the epidermis dorsal to the upper jaw and to the eyes. In the upper jaw region, HGC numbers were counted in three sections, each five sections apart; in the eye region, counts were made on 6-8 sections, each 10 sections apart. HGCs were recognised by containing a large number of small PAS positive granules. In addition, degenerating HGC material was present at some stages, showing up as single masses of PAS positive material in cells not otherwise stained.

(b) Proliferative activity was assessed by counting mitotic cells, when possible, in three different tissues - spinal cord, hindlimb mesenchyme and epidermis. The aim was to assess changes in proliferative activity in different tissues at different stages, rather than to make absolute measures of proliferation rate, a much greater task. The following counting procedure was used. For spinal cord, mitoses were counted on every 10th section in the region between the eyes (for stage 27 tadpoles, this involved counting seven sections, for stage 28 - eight sections, for stage 29 - nine sections) and a mean value per section was then calculated. Mitotic activity varies considerably along the spinal cord, but a mean value of this kind taken for one anatomical region gives a useful comparative measurement.

For hindlimb mesenchyme, which appears entirely undifferentiated at the stages examined here, mitoses were counted in the two limbs together every third or fifth section apart, depending on the sizes of the limbs, in order to obtain a mean value from four or five sections. At stage 27, limb buds were still very small and occurred in only a few sections. The mean number of mesenchyme cells per section was also calculated.

Epidermal mitotic activity was assessed at two locations: hindlimb epidermis of the same sections used for mesenchymal counts, and abdominal epidermis adjacent to a region used also to measure gut epithelial height.

(c) Intestinal lumen contents and intestinal epithelial cell yolk contents and height were assessed at different stages using three intestinal loops at a standard location. To compare different stages, drawings of the intestinal sections were made, using a x20 objective. Sample sections were also photographed.

RESULTS

LENGTHS, WEIGHTS AND MORPHOLOGICAL STAGES OF COMPLETE TADPOLES

Results from six separate clutches, each collected pre-hatching, are shown in Table 1 and a summary from one clutch in Fig. 1. Photographs of sample tadpoles from this clutch are shown in Fig. 2. The calculation of time since egg deposition requires some assumptions, since it was never known precisely when egg deposition occurred. Fortunately three clutches were found at very early stages of development, allowing the assumption that egg deposition occurred the previous night. In the table, the first night of development is ignored, and the number of development days counted from the next morning.

Hatching into the foam made by the adults occurred at stage 18-19 after two days development. This was determined by placing a few pre-hatching eggs in a small amount of foam, floating on the surface of water in a beaker, and noting when hatching occurred. By stage 21-22 tadpoles were found wriggling actively at the bottom of the foam mass. Tadpoles started making their own foam at about stage 25 (four days). It is clear from the morphological staging part of Table 1 that development continued for the following two days, to stage 28, judged in Gosner's system primarily by hind limb bud shape and dimensions (Fig. 3). After this, development became very slow. By 11-12 days, tadpoles had reached no farther than stage 28; by 19-20 days, stage 29, and by 28-29 days, stage 29-30. By comparison, if tadpoles were transferred to water with food as soon as they reached stage 28, they grew and developed very rapidly. Table 1 shows results from one such transfer, with stage 29 reached after 1 day and stage 29-30 after only two days. (A more detailed account of

Clutch	Time (days) since egg deposition [1]	Stage (Gosner)	Number measured	Wet Weight[2] (mg)	Total Length[2] (mm)	Body Length[2] (mm)
(1)	3	22	4	4.7 ± 0.3	-	-
Collected	4	25	3	6.3 ± 0.4	8.4 ± 0.5	-
2/7/91	5	27-28	3	8.3 ± 0.4	10.5 ± 0.3	3.8 ± 0.4
at cleavage	6	28	3	8.0 ± 0.5	10.1 ± 0.3	3.7 ± 0.1
stage	20	28-29	2	7.4 ± 0.3	10.3 ± 0.4	3.8 ± 0.2
	28	29	2	5.2 ± 0.3	9.2 ± 0.3	3.5 ± 0.2
(3)	3	22	2	4.5 ± 0.2	-	-
Collected	4	25	2	5.9 ± 0.6	8.1 ± 0.1	-
3/7/91	5	27-28	3	8.3 ± 0.3	10.5 ± 0.3	3.8 ± 0.1
at stage	6	28	3	9.4 ± 0.9	10.8 ± 0.7	4.1 ± 0.3
18/19	21	39-30	3	6.4 ± 1.3	9.6 ± 0.8	3.6 ± 0.3
(4)	3	21	4	5.8 ± 0.1	-	-
Collected	4	25	3	7.6 ± 0.3	9.3 ± 0.1	-
3/7/91	5	27-28	2	8.3 ± 0.4	10.5 ± 0.0	3.8 ± 0.0
at stage	6	28, 27-28	3	9.0 ± 0.9	10.8 ± 0.2	3.7 ± 0.0
19	29	29-30	4	4.1 ± 0.9	8.8 ± 0.8	3.1 ± 0.4
(6)	3	23	3	5.8 ± 0.3	-	-
Collected	5	27-28	4	9.1 ± 0.9	10.4 ± 0.5	3.9 ± 0.1
15/7/91	6	28	4	10.0 ± 0.6	11.2 ± 0.4	4.2 ± 0.1
at stage	12	28, 30	4	13.0 ± 4.9	11.8 ± 1.1	4.5 ± 0.5
19	19	29	4	12.2 ± 0.8	11.6 ± 1.0	4.5 ± 0.3
	28	29-30	2	6.2 ± 0.5	9.3 ± 1.0	3.6 ± 0.1
(6)	7	28	4	26.5 ± 3.7	13.8 ± 0.4	5.4 ± 0.3
Transferred to water with food, day 6	8	29-30	4	99.8 ± 9.5	19.0 ± 0.4	8.1 ± 0.3
(7)	3	23	2	5.6 ± 1.1	-	-
Collected	4	26	-	-	-	-
19/7/91	5	27-28	2	10.9 ± 0.1	11.2 ± 0.0	4.2 ± 0.3
at stage	12	28	4	9.8 ± 1.5	11.5 ± 0.7	4.2 ± 0.2
16	23	29	2	5.9 ± 1.6	-	3.4 ± 0.4
(16)	5	27-28	-	-	-	-
Collected	11	28	2	9.7 ± 2.1	11.3 ± 1.0	4.3 ± 0.4
31/7/91	19	29	4	7.3 ± 1.0	10.8 ± 0.4	3.9 ± 0.2
at cleavage stage	25	29	2	6.1 ± 0.8	9.7 ± 1.0	3.8 ± 0.1

TABLE 1. Growth and development of *L. fuscus* tadpoles kept in foam (with one group transferred to water with food).
[1] Time since deposition: given in days, starting from the morning after the night of oviposition.
[2] Mean ± SD.

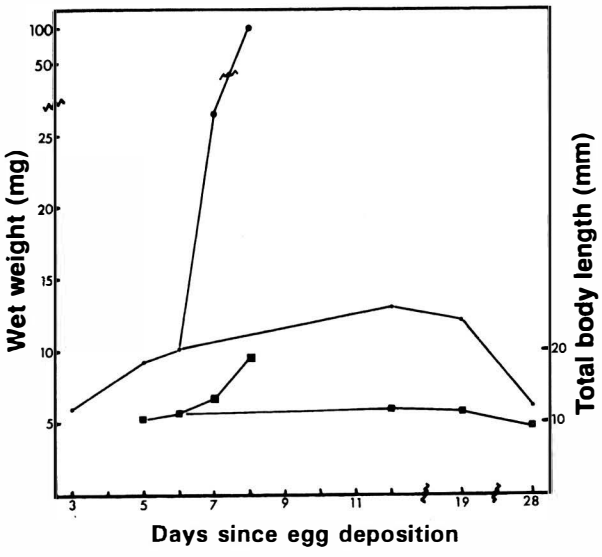

FIG. 1. Tadpole growth. Small symbols = tadpoles kept in foam; large symbols = tadpoles transferred to water with food. Circles = wet weights; squares = total body length; data from clutch 6, Table 1. Each point represents a mean value for several tadpoles.

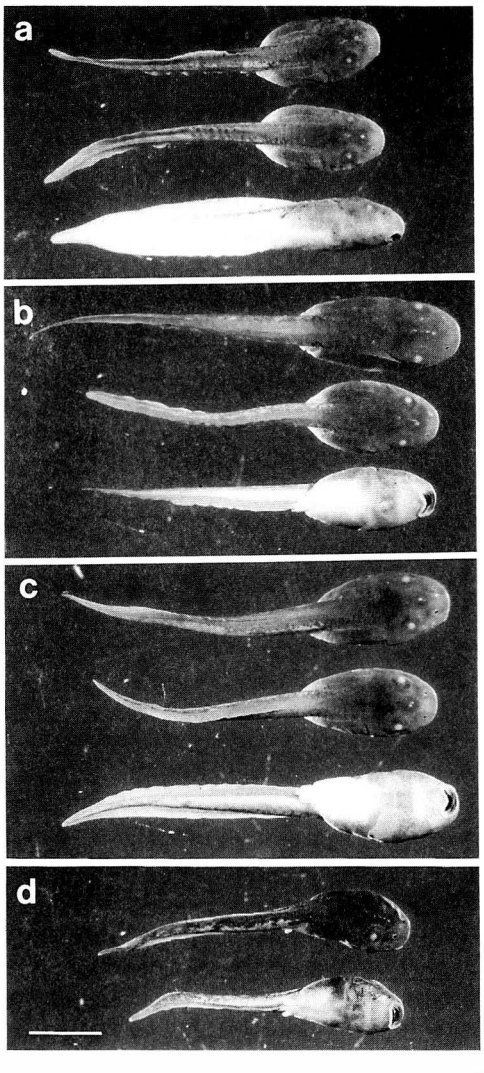

FIG. 2 Whole *L. fuscus* tadpoles from a single clutch of eggs, kept in foam for different times. (a) 5 days after deposition, (b) 12 days, (c) 19 days, and (d) 28 days. Scale line = 2mm

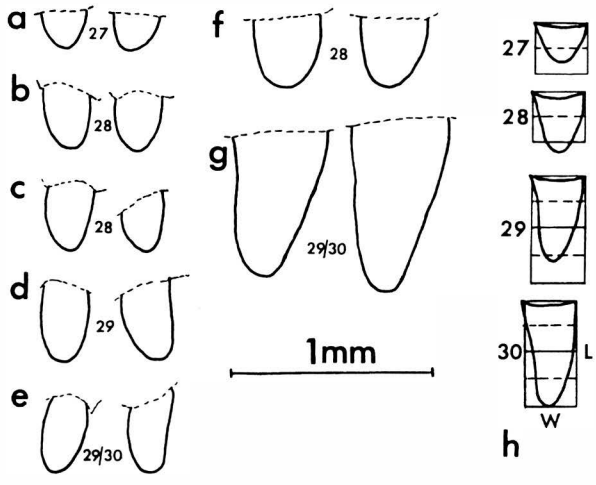

FIG. 3. Camera lucida drawings of hindlimb buds of *L. fuscus* tadpoles from a single clutch of eggs with Gosner stage numbers. (a)-(e) kept in foam for 5, 6, 12, 19, and 28 days post egg deposition, respectively. (f)-(g) tadpoles transferred to water with food at 6 days and fixed after 1, 2 days, respectively. (h) for reference, Gosner (1960) stage hindlimb bud drawings (not to scale). At stage 27, l ≥ ½ x w; stage 28, l ≥ 1 x w; stage 29, l ≥ 1½ x w; stage 30, l = 2 x w. on this basis, buds (e) and (g) are both on the borderline between stages 29 and 30, but very different in size.

such transfer experiments is given in a separate paper - Downie, 1994). Sample limb-bud drawings are shown in Fig. 3. It was clear that though limb-bud shape progressed through to stage 29-30 in tadpoles kept in foam, these limb-buds were much smaller than those of the equivalent morphological stage in tadpoles transferred to food with water. Indeed, in foam, limb bud shape seemed to progress not by relatively greater growth in length compared to width, but by narrowing width while keeping length the same.

The tadpole measurements given in Table 1 show that wet weight and length increased until stage 27 to 28 at 5-6 days. Lengths and weights remained fairly stable for the next week or so, then declined: in four clutches maintained in foam for 23-29 days post deposition, the final wet weight was 49-68% of that at 6 days. Length decreases were less marked but were consistent. Dry weight changes over 22-23 days in foam are shown in Table 2. In this sample, wet weight at 28 days was 54% of that at 5-6 days. Dry weight declined proportionately even more, to 36%, suggesting that wet weight

Tadpole stage	Number measured	Mean wet weight±SD (mg)	Mean dry weight±SD (mg)
5-6 days after deposition	8	8.0 ± 0.6	1.4 ± 0.2
28 days after deposition	7	4.3 ± 1.2	0.5 ± 0.1

TABLE 2. Comparison of wet and dry weights for *L. fuscus* tadpoles at two stages, both kept in foam.

measurements underestimate the loss of tissue mass when tadpoles spend extended times in foam.

Numbers of tadpoles measured in each clutch were small, because tadpoles were also needed for growth experiments, but the trends were consistent.

One anomalous result occurred. In clutch 6, one tadpole kept in foam had reached stage 30 by 12 days, and was much larger than normal. It is not known whether this was the result of an unusually large egg, or possibly from cannibalistic behaviour in the foam. Very occasionally, tadpoles kept in the foam were found with shortened tails, presumably the result of cannibalism.

In six clutches where deposition time was known, tadpoles maintained in foam eventually died. Survival times post-deposition ranged from 19-33 days (mean 27.5 days).

Tadpoles kept long-term in foam differed markedly in appearance from those at the start (Fig. 2). In particular, the abdomen and fins shrank considerably in size.

HISTOLOGICAL RESULTS

Hatching gland cell (HGC) numbers. In anurans, larval hatching is facilitated by the secretion of lytic enzymes by a group of HGCs located as a patch in frontal region epidermis, extending as individual cells some way back along the dorsal mid-line: after hatching, HGCs regress, eventually disappearing altogether (Yoshizaki & Katagiri, 1975). HGCs are readily recognised in section as large flask-shaped cells, packed full of PAS - positive granules. When HGCs regress, fragments of PAS - positive material remain in the epidermis of the HGC zone for some time.

Since HGC regression is a developmental process occurring around the time when developmental arrest happens in *L. fuscus*, it seemed worthwhile to discover what effect arrest had on this process, if any.

The hatching stage for both *L. fuscus* and *P. pustulosus* was determined by withdrawing eggs from foam nests and floating them on the surface of water. In *L. fuscus*, hatching occurred at stage 18-19 and in *P. pustulosus* at stage 23-24 (Gosner, 1960, gives the normal hatching range as stages 17-20 for a wide variety of tadpole species).

HGC numbers were counted for *L. fuscus* tadpoles kept in foam, and transferred to water, and for *P. pustulosus* (Table 3).

The results for *L. fuscus* in foam show that HGCs remained abundant through to stage 27 (about 3 days after hatching). When stage 27 tadpoles were transferred to water with food for two days, they progressed to stage 28, and all HGCs disappeared. However, if the same batch of tadpoles was kept in foam for the same time, development proceeded hardly at all and HGCs remained abundant. After a further two days in foam, these tadpoles reached stage 28, but still retained some HGCs, and a few HGCs remained present after a further one and three days in foam. However, after two or three weeks in foam all HGCs had disappeared.

One odd feature of the *L. fuscus* results is that the number of HGCs appeared to increase in the jaw region between stages 24 and 27/28. This seems unlikely to be the case, given the accepted function of HGCs. The result may be due to differential growth in the frontal area, moving some HGC posteriorly, but only a detailed cell count can clarify this point.

By comparison, HGCs disappeared earlier in *P. pustulosus*: all were gone by stage 28/29, 6 days after hatching and even four days after hatching, numbers were small compared to *L. fuscus* at the same stage. This is despite the fact that hatching in *P. pustulosus* is at a later stage than in *L. fuscus*.

Proliferative activity in arrested and non-arrested tadpoles. Results for mitotic counts are given in Fig. 4. As is made clear in the Materials and Methods section, counts for the different tissues are for different ana-

Days after oviposition	Gosner stage	Frontal area	Jaw region	Eye region
(a) *L. fuscus* tadpoles kept in foam				
4(6)	24	many	7.2 (4.0-13.0)	3.1 (0.6-4.8)
5(4)	27	many-very few	15.8 (5.7-25.0)	0.05 (0-0.1)
6(3)	27-28	many-few	14.3 (11.0-18.0)	0
8(3)	28	few-none	4.0 (0.3-8.3)	0
9(3)	28	few-very few	1.7 (0-4.3)	0
11(3)	28	few-very few	0.9 (1.0-1.7)	0
16(2)	28	none	0	0
23(2)	28	none	0	0
(b) *L. fuscus* tadpoles transferred to water with food for 2 days				
5(2)	28	none	0	0
(c) *P. pustulosus* tadpoles				
4(4)	24	many-none	6.8 (3.0-8.7)	1.3 (0.3-3.5)
6(4)	27	very few-none	1.2 (0-3.3)	0.03 (0-0.1)
8(2)	27-28	none	0	0

TABLE 3. Hatching gland cell (HGC) no. in early *L. fuscus* and *P. pustulosus* tadpoles. No. of tadpoles sampled shown in parentheses. In the jaw region, HGC no. for each tadpole were the mean from 3 sections; in the eye region, from 6-8 sections. In the frontal region, HGCs were assessed as many, few, very few or none. Figures given are means and ranges.

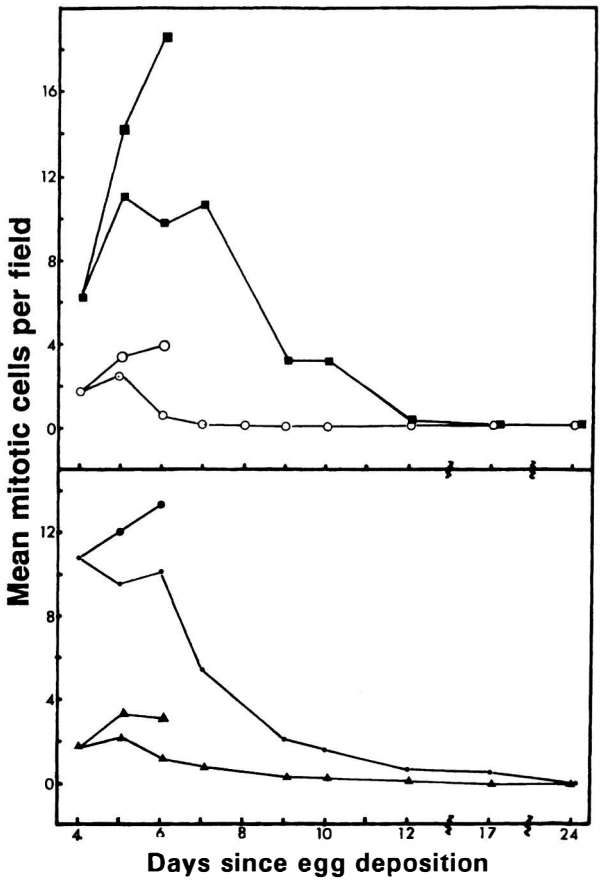

FIG. 4. Mitotic counts from different *L. fuscus* tadpole tissues. Small symbols = tadpoles kept in foam; large symbols = tadpoles transferred to water with food. Each point represents a mean value from several tadpoles. Filled circles = brain; triangles = limb epidermis (cells in field - 90); squares = limb mesenchyme (cells in field -500); open circles = abdominal epidermis (cells in field - 200).

(a) *Tadpoles kept in foam*

Days after oviposition	*n*	Height in μm : mean ± SD	
		Short side	Long sides
5	6	107 ± 14	117 ± 13
6	6	86 ± 8	101 ± 9
11-12	6	54 ± 9	51 ± 11
19-21	6	45 ± 6	43 ± 6
28	4	31 ± 10	33 ± 7

(b) *Tadpoles transferred 4 days after oviposition*

No. of days in water with food	*n*	Height in μm : mean ± SD	
		Short side	Long sides
1	3	58 ± 2	69 ± 11
2	4	51 ± 5	49 ± 12
3	4	59 ± 9	61 ± 5

TABLE. 4. Intestinal heights. Measurements from intestinal loop L in Fig. 1. For each specimen the highest point was measured on the short side of the triangular loop and on the two longer sides. *n* = no. of tadpoles measured.

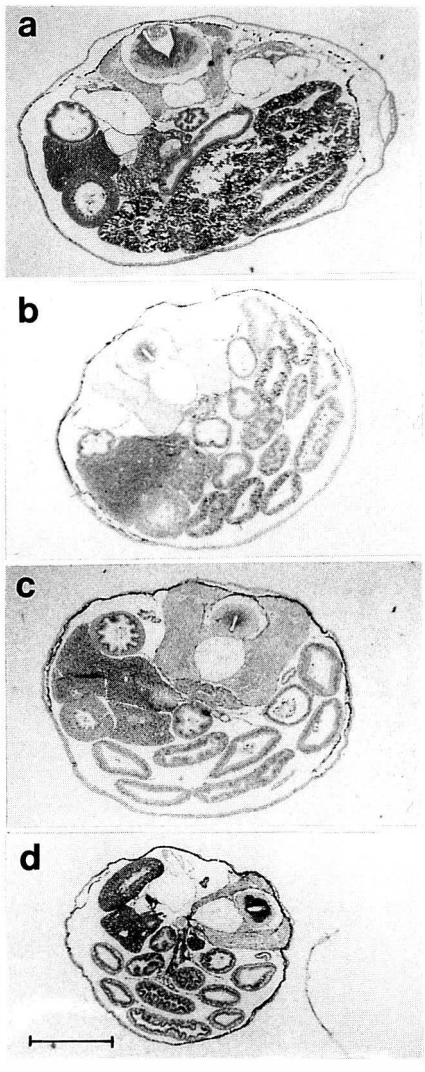

FIG. 5. Low power micrographs of haemalum and eosin-stained sections taken through the abdominal area of *L. fuscus* tadpoles from the same clutch of eggs, kept in foam for different times. (a) 5 days after egg deposition; (b) 12 days; (c) 19 days; and (d) 28 days. This region of the abdomen was used for measuring epidermal mitotic activity, and the gut area labelled L was used to determine intestinal epithelial height. Scale line = 0.5mm.

tomical areas: they are not made for a particular number of cells, and therefore cannot be compared directly. Given this limitation, some clear trends emerge.

In tadpoles kept in foam, proliferative activity remained high for about 2 days after the tadpoles started to make foam (stage 25). During this time, they progressed to stage 28. Thereafter, proliferative activity declined at different rates in different tissues. A small amount of activity was present in the brain and spinal cord even after 18 days development, whereas no activity was detectable in abdominal epidermis after only 8 days. By 25 days, no tissue showed mitotic activity.

If tadpoles were transferred to water with food as soon as they started to make foam, mitotic activity continued, at a slightly increasing rate, and stage 28-29 was reached after only two days, compared to over a week if tadpoles were kept in foam.

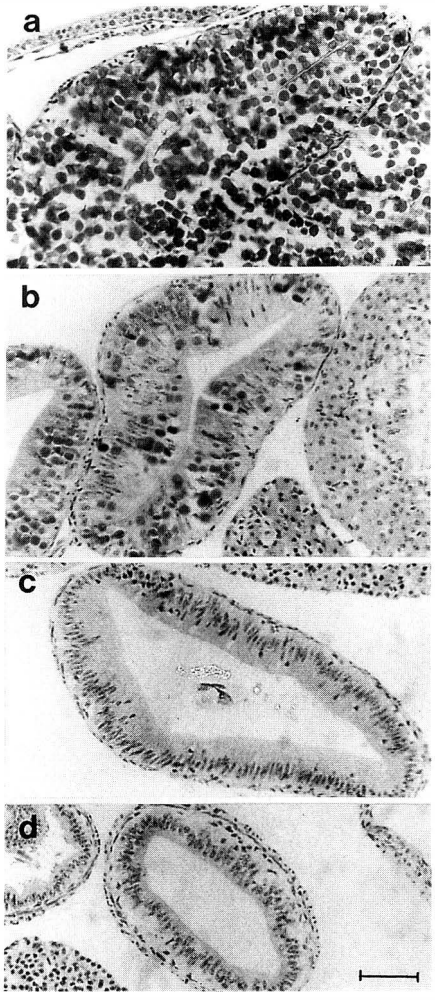

FIG. 6. High power micrographs of the gut sections labelled in Fig. 1. (a) 5 days after egg deposition - cells tall and packed with yolk granules; lumen occluded. (b) 12 days - cells lower, yolk still fairly abundant; lumen more open. (c) 19 days - cells lower still,one or two yolk granules only; lumen more open. (d) 28 days - cell height much reduced, yolk absent; lumen wide open. Scale line = 50 μm.

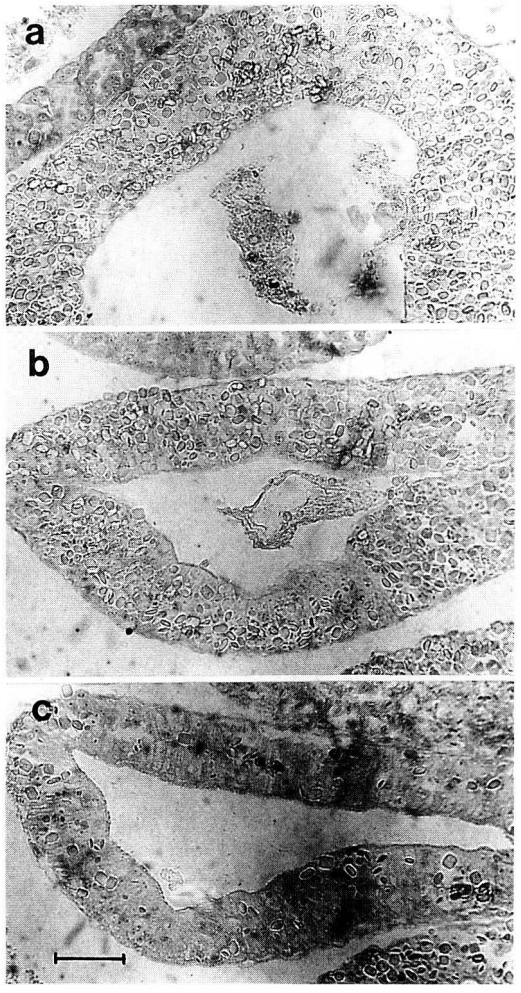

FIG. 7. High power micrographs of gut sections, equivalent to those labelled in Fig. 1, but from *L. fuscus* tadpoles transferred to water with food at stage 25 for (a) 1 day, (b) 2 days, and (c) 3 days. Scale line = 50 μm.

Intestinal cell height and yolk content. Camera lucida drawings of three intestinal loops (Fig. 5) were made from serial sections of tadpoles kept in foam for 5, 6, 7, 12, 19 and 28 days post egg deposition. For comparison, one batch of tadpoles at stage 25 (four days post deposition) was transferred to water with food, and fixed after one, two and three days growth. Sections of the intestines of these tadpoles were examined in the same way as those taken from foam. Representative sections are shown in Figs. 6 and 7. Quantitative results are shown in Table 4. The growth and development of the tadpole gut is complex, with different parts proceeding to new stages at different times: a full report on this is beyond the scope of this paper. However, comparing sections at standard locations, at different stages, from tadpoles kept in foam and those transferred to water with food, some clear trends emerged.

In tadpoles kept in foam, gut epithelial height remained high during days 5 and 6 post-deposition, with very little sign of yolk platelet diminution. By 11-12 days, epithelial height was considerably reduced as was yolk content. Yolk content at this stage was very variable and patchy along the length of the gut, but was always less than at days 5 and 6 and more than at days 19-21. By days 19-21, epithelial height was further reduced, as was yolk content. One 19 day specimen contained a few small yolk particles. All the others had none. By day 28, epithelial height was reduced even more: no yolk particles were present.

In tadpoles transferred to water with food at day four, epithelial height was considerably reduced after one day, compared to tadpoles kept in foam. The lumen was wide open and full of contents. Epithelial cell yolk content was not noticeably different from tadpoles kept in foam. There was little change after two days in water, but, by three days, yolk content was noticeably less, to about the level found in tadpoles kept in foam for 11-12 days.

It is worth pointing out that the guts of tadpoles kept in foam were not empty, at least until days 19-21. As

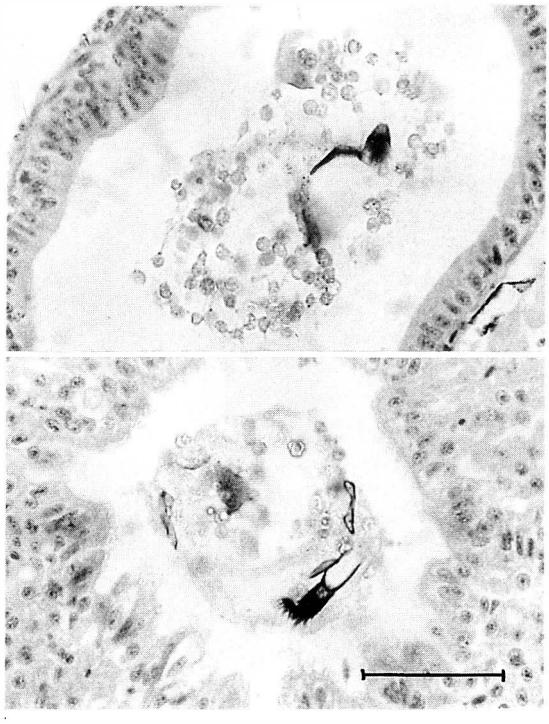

FIG. 8. High power micrographs of *L. fuscus* tadpole intestine sections to show tadpole teeth (dark, jagged structures) and single-celled micro-organisms (small round structures) amongst contents. This tadpole had been kept in foam two weeks after hatching. Scale line = 50 μm.

well as some amorphous contents, two kinds of particle were common (Fig. 8). The first were dark brown/black structures which turned out to be shed tadpole teeth. The others were numerous small round cells, 4-6 μm diameter. The teeth were presumably shed by all tadpoles in the foam, since they appear to be continuously produced in rows with the most distal ones being lost, like skin squames in mammals. The round cells may be yeasts or algae either living in the gut or ingested from foam. Tadpoles kept in foam 28 days had guts which appeared to be empty.

DISCUSSION

The results presented document the developmental state of *Leptodactylus fuscus* hatchlings when they remain long term in the nest, rather than entering water. In this species, eggs are laid in burrows close to the sites of temporary pools, or at the sides of drainage ditches: the eggs are deposited in a mass of foam, and the burrow entrance is then closed with mud. When the pool or ditch floods, the soft mud at the entrance breaks open and the tadpoles emerge into the water. It has been known for some time (Kenny, 1969; Martins, 1988) that the tadpoles hatch into the foam and may survive there for several weeks if heavy rain does not occur. More recently, it has been established (Downie, 1984; 1989) that some time after hatching, the tadpoles start to make a new kind of foam which replaces the original nest foam, and that it is in this that the longterm survival of the tadpoles occurs.

In this paper, I show that hatching occurred at Gosner stage 18-19, two days after the night of egg deposition. Tadpoles started to make their own foam at stage 25, two days later. They continued to develop for about two days more, reaching stage 28. Any further development, judged by limb bud morphology was very slow. By contrast, tadpoles transferred to water with food developed rapidly. Tadpole clutches recorded in this study showed a mean survival time in foam of 27.5 days post egg deposition, or 23.5 days after the onset of foam-making. Tadpoles long-term in foam did not remain unchanged, however. They reached a maximum length and wet weight after 5-6 days of development: after this, they declined - towards the end of the survival period, length and wet weight were about half the maximum attained, and dry weight even more diminished.

In a study of two other 'fuscus' group leptodactylids, *L. prognathus* (*latinasus*) and *L. bufonius*, Pisano & Del Rio (1968) suggested that in nest foam, tadpole growth was inhibited but that morphogenesis and differentiation continued normally. Pisano & Del Rio's conclusions were based on comparisons of growth and development between tadpoles kept in foam and others transferred to water without food at the tail-bud stage (Gosner, stage 17). Unfortunately, they did not recognise the possible importance of the new foam made by the tadpoles (Caldwell & Lopez's [1989] report from a second 'fuscus' group species, *L. mystaceus*, suggests that this behaviour is likely to be a group characteristic) nor did they use a recognised staging system to determine the progress of development. Further, their growth results were presented only as lengths with no numbers given nor any statistical treatment. Pisano & Del Rio demonstrated a rapid increase in length when tail bud stage larvae were transferred to water. However, thereafter, the growth rates of their 'water' and 'foam' groups were essentially the same for the next few days, with growth in foam then ceasing 4-5 days after the tail bud stage. If we assume that the initial increase in length of tail bud larvae in water was due to tissue hydration, these results are essentially the same as mine: in foam, development continued until soon after the tadpoles started making their own foam, and then essentially ceased. Pisano & Del Rio also carried out some histological analysis of their specimens, concentrating on chromatophore distribution and gut morphogenesis, but in my view, these were inadequate to support their contention that differentiation continued in foam, and that only growth was inhibited.

The measurements of development reported here were chosen to test whether progressive developmental processes are inhibited in foam. The following processes were examined:

(1) Hind limb bud morphology is the main criterion for assessing the development of feeding stage tadpoles (Gosner, 1960). In foam, limb buds progressed slowly from stage 27 (5 days after deposition) to stage 29 (19

days after deposition) but this progression involved reshaping of existing tissue whereas tadpoles transferred to water with food much more rapidly attained stage 29 by means of more rapid growth in limb length than in width. It is clear, therefore, that in foam, limb bud development was essentially arrested.

(2) Hatching gland cell (HGC) numbers were assessed as an example of a degenerative change that normally occurs over the period under study. Again, HGC degeneration did occur, but much more slowly in foam than in tadpoles transferred to water, or in tadpoles of another leptodactylid.

(3) Mitotic activity was measured in three tissues: hindlimb mesenchyme, central nervous system and epidermis. In tadpoles transferred to water with food, mitotic activity continued in all these tissues. In tadpoles maintained in foam, mitotic activity declined and eventually ceased entirely in all tissues.

(4) Intestinal epithelial morphology and yolk content measurements showed that epithelial cell shape change and yolk particle utilization were both slowed considerably in foam compared to tadpoles transferred to water with food.

From all these measures, I conclude that soon after *L. fuscus* tadpoles started making foam, overall growth ceased and progressive developmental processes slowed down drastically. It is important to note that these changes occurred while the gut still contained a considerable amount of yolk, though precise measurement of the amount would be difficult.

These observations suggest that before yolk is fully used up, tadpoles that have not entered water slow down development so as to preserve food reserves for a possibly lengthy period in the foam nest. Once yolk reserves have been fully utilized for metabolic needs, larval tissues are catabolised, as shown by the steep decline in dry weight in tadpoles kept longterm in foam. In one clutch, yolk was entirely used up by 20 days post deposition, but tadpoles survived till 28 days. I have not attempted to measure the metabolic rate of tadpoles kept in foam, though this would be worthwhile. However, these tadpoles do not have the option of shutting down all metabolic processes, since they require to maintain the foam nest and this involves activity (Downie, 1989).

It is instructive to compare these observations with the myobatrachid genus *Pseudophryne* (Bradford & Seymour, 1985; 1988). In *Pseudophryne bibroni* eggs are laid on land in moist soil. At a normal ambient temperature of around 12°C, they develop in 39 days to Gosner stage 26-27, when they are capable of hatching. Hatching, however, only occurs if the eggs are then subjected to prolonged flooding. In the absence of flooding, the larvae remain unhatched for up to three months. During this prolonged unhatched period, development greatly slows down, with stage 28 being eventually reached: metabolic rate, measured by oxygen consumption declines a little from the peak attained around 39 days, but then reaches a plateau: yolk reserves are eventually used up and body dry mass then starts to decline. From all these features, developmental arrest in *P. bibroni* is clearly very similar to that in *L. fuscus*, the main differences being in timescale (related to temperature) and in the fact that arrest occurs before hatching in *P. bibroni* and after hatching in *L. fuscus* but at approximately the same developmental stage.

I have used the term 'developmental arrest' to describe the state that *L. fuscus* tadpoles in foam, or unhatched *P. bibroni* are in, and it is worth discussing briefly what sort of state this is. Examples of developmental arrest occur throughout the animal kingdom and are very varied in their nature (see Clutter, 1978 for useful reviews). Ewert (1991) has recently reviewed the kinds of arrest that occur in reptiles and birds and defined these as diapause, delayed hatching and embryonic aestivation. In a true diapause, environmental conditions may be satisfactory for development to proceed, but development is arrested and requires a definite stimulus to restart. True diapauses are stage specific - they cannot occur simply at any time during development. In any particular species, diapause may be facultative (occurring in response to environmental conditions) or obligate (occurring irrespective of conditions). In *L. fuscus*, developmental arrest meets some, but not all of the characteristics of diapause. Arrest occurs at a definite stage of development (prolonged survival out of water at later stages does not occur, though short-term survival does (Downie, 1984) but is certainly not obligate. The means by which arrest is maintained and the stimuli for its beginning and ending are discussed in a separate paper (Downie, 1994).

It seems obvious that the function of the arrest period in *L. fuscus* is to allow tadpole survival until rain falls, providing conditions for further development. Two questions arise: first, how long can tadpoles survive in their nests in the field? In the laboratory, tadpoles survived about four weeks from the time of egg deposition, but this was at 25-28°C, at constantly high humidity and in the absence of predators. Long term survival in the field may be affected by differences in all these factors, and this requires investigation. Secondly, how fit for further development are tadpoles that have survived several weeks in foam? The loss of weight that occurred late in the survival period suggests that tadpoles may be in poor condition, even if rain then falls; this requires investigation too.

A brief discussion of possible cannibalism among tadpoles in foam is necessary. The intestines of tadpoles in foam were not empty, except towards the end. Contents included small round cells and tadpole teeth fragments. The literature suggests that the round cells may be yeasts (*Candida*-Steinwascher, 1979) or unpigmented algae (*Prototheca*-Beebee, 1991): their possible effect is discussed by Downie (1994). This suggests that the tadpoles may ingest material - mostly foam - from their surroundings. Downie (1994)

showed that *L. fuscus* eggs added to heaps of foam-making tadpoles were rapidly consumed: no doubt these can provide nutrition and, in natural conditions, this behaviour would rid the nest of non-developing and possibly diseased eggs that might provide a risk of infection to the tadpoles. If the tadpoles are able to feed in foam, cannibalism might be expected, especially tail tip nibbling. However, nibbled tail tips appeared to be rather uncommon in foam nests, and total numbers of tadpoles remained remarkably constant over several weeks (Downie, unpublished observations) suggesting that cannibalism was rare in this situation, despite its possible attraction as a survival strategy for individual tadpoles. However, an unusually large tadpole was found in one clutch possibly the result of cannibalism; this also invites further investigation.

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DEVELOPMENTAL ARREST IN *LEPTODACTYLUS FUSCUS* TADPOLES (ANURA: LEPTODACTYLIDAE). II: DOES A FOAM-BORNE FACTOR BLOCK DEVELOPMENT?

J. R. DOWNIE

Department of Zoology, University of Glasgow, Glasgow G12 8QQ, UK

Several experiments were performed to test the hypothesis that foam made by hatched *Leptodactylus fuscus* tadpoles contains an inhibitor that maintains them in a state of developmental arrest. The results did not support the hypothesis. Tadpole-made foam did not inhibit the development of earlier stage *L. fuscus*, later stage *L. fuscus* or tadpoles of another species, *Colostethus trinitatis*, nor did removal from foam in itself release tadpoles from developmental arrest. Developmental arrest was found not to be obligate: tadpoles transferred to water at pre-arrest stages developed continuously through to later stages. Preliminary evidence suggested that transfer to water alone, irrespective of the presence of food, allowed tadpoles to bypass the arrest stage at least partially, possibly using their yolk reserves to continue development. The possibility that developmental arrest is mediated via *Candida* or *Prototheca* infection is briefly discussed.

INTRODUCTION

Frogs of the *Leptodactylus* '*fuscus*' group (Heyer, 1978) lay their eggs in foam nests in burrows on land, near sites of temporary pools, but generally in advance of heavy rains. In the case of *Leptodactylus fuscus*, if no rain falls, development proceeds past hatching and the larvae start to make a new kind of foam within which they can survive for several weeks (Downie 1984, 1989). Caldwell & Lopez (1989) reported similar foam-making behaviour by *L. mystaceus*, another member of the 'fuscus' group, and this may therefore be a general characteristic of the group. Soon after the tadpoles start making foam, development slows down drastically. Downie (1994) has shown that foam-making begins at Gosner (1960) stage 25 - four days after the night of egg deposition; that development proceeds for two more days to stage 28, then essentially stops with tadpoles reaching stage 29 after a further 13 days or so in foam. In contrast, tadpoles transferred to water with food as soon as they reach stage 28 attain stage 29 after only two days. The state of the tadpoles during developmental arrest has been characterized in terms of declining mitotic activity in several tissues, slowed hatching gland regression, slowed limb bud morphogenesis and slowed yolk utilization, all compared to tadpoles transferred to water and fed.

An obvious question arises: what brings about developmental arrest? Pisano & Del Rio (1968) reported developmental arrest - which they took to involve cessation of growth but not of morphogenesis and differentiation - in two further species of the 'fuscus' group, *L. prognathus* (= *L. latinasus*; Heyer, 1978) and *L. bufonius* and suggested that the nest foam made by the adult frogs contains a growth inhibitor. Pisano & Del Rio (1968) were unaware of the possibility of the new kind of foam made by the tadpoles. Wassersug (1986) used the correlation of the formation of larval-produced foam with the beginning of developmental arrest, to suggest that the foam produced by the tadpoles contains a development inhibitor, specifically suggesting that prostaglandins may be involved.

In this paper, I report tests of the idea that tadpole-produced foam contains development inhibiting activity in *L. fuscus*. The results give no support to Wassersug's proposal. The results of further experiments suggest other ways in which developmental arrest may be maintained.

A secondary question is whether developmental arrest is obligate (as diapause is in some kinds of animals) or facultative, depending on conditions. Results from experiments where tadpoles develop under different conditions show that developmental arrest is facultative in this species.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF TADPOLES

Foam nests of *L. fuscus* were collected from burrows around the margin of a temporary pool site on the University of West Indies campus at St Augustine, Trinidad and from the banks of drainage ditches in St Augustine and at Piarco Road (near the airport) during June-August 1987, 1989 and 1991. On collection, a few eggs or tadpoles were removed for staging using Gosner's (1960) normal table. Foam nests were maintained in the laboratory on the surface of moist tissue in closed 250 ml polythene tubs. Laboratory temperature ranged from 27-29°C during this work. Since it was found that the tadpoles continued to make foam both in the light and in the dark, no attempt was made to control light levels. Every week or so, tadpoles in foam were removed to fresh tubs to avoid the build-up of possibly deleterious waste products.

When later stage *L. fuscus* tadpoles were required, tadpoles were removed from foam nests to glass aquarium tanks and fed *ad lib* with aquarium fish food. For comparative purposes, tadpoles of another species were used. These were *Colostethus trinitatis*, collected from Tamana cave in central Trinidad. *C. trinitatis* tadpoles are a useful comparator because, like *L. fuscus*, they start development on land. After hatching, they are carried by the male frog to water, but they may remain on his back, out of water, for several days. *C. trinitatis* tadpoles were maintained in glass tanks and fed in the same way as later stage *L. fuscus*. For general accounts of these two species, see Kenny (1969), and for nomenclature changes Harding (1983).

Experiments on the Potential Growth and Development Effects of Foam

Foam-making tadpoles at stage 28 were divided into groups of 10-20 (according to availability) in separate tubs and left at least one day to make ample heaps of foam. To test the inhibitory effects of this foam, three kinds of experiment were set up.

Experiment 1. Leptodactylus fuscus eggs or larvae from newly collected clutches at stages earlier than foam-making (i.e. prior to stage 25) were added to foam heaps. Foam-making tadpoles were not removed because their presence was necessary to maintain the foam heap.

At the time of addition, the stages of the added eggs or larvae were recorded. They were then removed from the foam heaps one or two days later and their developmental stages again recorded. At these times, the stages of controls (from the same clutches, but allowed to develop normally in their own foam nests) were also recorded. In addition, since the removal of eggs or larvae from the foam nest might have had a damaging effect (for example, from handling) two other controls were set up: eggs or larvae were removed from foam nests and incubated in water, or on the surface of damp tissue paper.

Experiment 2. Leptodactylus fuscus tadpoles, past stage 30, were measured and staged, then added singly to foam heaps. After two days, these were removed and remeasured. For measurements, these tadpoles were anaesthetized in 50 $\mu g\ ml^{-1}$ MS222 (Sandoz), then total length measured to 0.1 mm with calipers (Camlab); tadpoles were then allowed to recover in water before being placed in a foam heap. Since this experiment involved the removal of test tadpoles both from food and water, the following controls were necessary: tadpoles kept for the same time in water without food; tadpoles kept for the same time in water with food; and tadpoles kept out of water, on the surface of moist tissue in tubs like those used for the foam treatment. These were anaesthetized and measured in the same ways as experimental tadpoles.

Experiment 3. To test for the specificity of any possible inhibitory effect of foam, *Colostethus trinitatis* tadpoles were treated in the same way as past stage 30 *L. fuscus*. Previous work (Downie, unpublished) had shown that both *L. fuscus* and *C. trinitatis* tadpoles survive well for some days out of water on a damp substrate.

Experiments on the Release of Tadpoles From Developmental Arrest

To test the conditions under which foam-making tadpoles are released from developmental arrest and whether developmental arrest is obligate or facultative, tadpoles at different stages were removed from foam and their growth and development assessed under several treatments. The stages used were (a) start of new foam-making (Gosner stage 25), approximately 4 days after egg deposition; (b) start of developmental arrest (Gosner stage 28), approximately 6 days after deposition; (c) after 6 days developmental arrest (still Gosner stage 28), approximately 12 days after deposition.

The treatments used were (a) tadpoles removed from foam, washed and placed individually on the surface of moist tissue paper in closed 250 ml polythene tubs. The paper was fully saturated with water, but there was no free water for tadpoles to swim in. Tadpoles kept several centimetres apart under these conditions make little if any foam (Downie, 1990); (b) tadpoles removed from foam and placed in 1500 ml of dechlorinated tap water in 2 litre polythene tubs, with no addition of food: no aeration was necessary because of the small sizes and numbers of tadpoles, and the short duration of the treatment - 2 days maximum; (c) tadpoles removed from foam to water, as above, but with the addition of a small quantity of powdered fish food flakes.

Tadpoles from each treatment were removed at (generally) daily intervals, fixed and stored in Bouin's fluid, then later measured and staged. Total length was measured using a Wild M5 binocular microscope with calibrated eyepiece graticule at x6 objective magnification. Body length, defined as the distance from snout to hind-limb bases was measured in the same way. Limb buds were examined at higher magnification and some were drawn using a Wild drawing tube: these observations were the main criterion for assessing the Gosner (1960) developmental stage. Each tadpole was weighed in two ways: first, tadpoles were damp-dried on tissue paper, then weighed immediately to give wet weights; next, tadpoles were dried to constant weight in an oven, then reweighed to give dry weights. All weighings were on a Sartorius Research balance to 0.1 mg. In one experiment, tadpoles were to be sectioned after measuring: these were wet-weighed only.

As controls, tadpoles kept in foam were fixed at the same times as experimentals and staged and measured in the same ways. All treatments and controls were carried out in the same laboratory conditions.

To measure mitotic activity, tadpoles from one experiment were wax embedded after taking basic measurements, serial sectioned at 7 μm and stained with haemalum and eosin. Mitotic activity was counted at three locations: brain, hindlimb bud mesenchyme

and epidermis by the same procedure as Downie (1994), giving a comparison of mitotic activity in different treatments.

RESULTS

ADDITION OF PRE-FOAM-MAKING LARVAE TO FOAM HEAPS

The rationale of this experiment was to expose early larvae to tadpole foam to test whether they continued to develop or were inhibited. This was not a particularly easy experiment to set up as it required finding early stage nests at the same time as healthy foam-making tadpole clutches were available in the laboratory. Only one such experiment could be set up in season 1989, but, fortunately, several replicates were possible in season 1991. Eggs or larvae were added at three stages: late pre-hatching, stage 20 and stage 23 (approximately 1, 2 and 3 days after deposition respectively).

In the case of 16 pre-hatching eggs, set up in four separate trials, all had disappeared after one day, leaving a few fragments only. Presumably these eggs were eaten by the foam-making tadpoles. A similar result occurred with 49 stage 20 embryos, added in 11 separate trials. In seven of these trials, the foam heaps stayed more or less in the same place and added embryos had either disappeared after one day, or a few fragments remained. In the remaining four trials, the foam-making tadpoles moved position. Any added embryos not already consumed were left stranded and were found dead after one day. Control pre-hatching eggs or stage 20 embryos incubated on damp tissue paper for one day also died in most cases, whereas those isolated from their foam nests and incubated in water developed after one day to approximately the same stage as those left in their foam nests.

A different result occurred with 38 stage 23 larvae added in eight separate trials. Of these, 26 developed through to stage 27/28 after two days, the same stage as controls left in their foam nests, or incubated in water. The remaining 12 died or were eaten, most of these being in two tubs where the foam-making tadpoles stopped making foam during the experiment. No added tadpoles showed any sign of inhibited development.

EFFECTS OF FOAM ON LATER STAGE TADPOLES

To test the inhibitory effects of tadpole-foam on stage 30+ *L. fuscus* and *C. trinitatis* tadpoles, after measuring, tadpoles were added as individuals to active foam-making *L. fuscus* heaps in small tubs and left for two days, when they were re-measured. Three control groups were used: tadpoles in water, with or without food; and tadpoles on the surface of moist tissue paper. The results are shown in Table 1. *L. fuscus* tadpoles, as might be expected, stopped growing when deprived of food. Out of water on moist tissue paper, they decreased in length. In foam, they also decreased in length by a mean percentage not significantly different from those on moist tissue paper. *C. trinitatis* tadpoles somewhat surprisingly did not grow significantly in water with or without food during the time of the experiment. On moist tissue paper, they decreased in length. In foam, their length loss was not significantly different from that on moist tissue paper. Out of water, these later stage tadpoles were quite active, especially those of *C. trinitatis*, and those placed in foam heaps did not always stay in the foam; they were, however, in small tubs and must have been in contact with the foam much of the two days. The results clearly show that exposure to foam has no more inhibitory effect on either tadpole species than simply depriving them of food and keeping them in humid conditions out of water.

RELEASE OF TADPOLES FROM DEVELOPMENTAL ARREST

To test the conditions under which tadpoles are released from developmental arrest, and to discover whether arrest is obligate or facultative, tadpoles at three stages (start of foam-making, start of developmental arrest and after six days of developmental arrest) were removed from nest foam and treated in

Species	A Water,no food		B Water,with food		C On moist tissue		D In tadpole-foam		F-ratio
	n	%ch. ± SD	n	%ch. ± SD	n	%ch. ± SD	n	%ch. ± SD	
L. fuscus	20	-0.4±2.8^a	20	+10.3±5.9^b	19	-14.1±3.8^c	20	-15.0±5.5^c	$F_{3,75}$=179.89***
C. trinitatis	18	+0.6±2.3^a	16	+0.7±3.7^a	18	-9.5±3.3^b	14	-10.5±2.4^b	$F_{3,62}$=39.90***

TABLE 1. Mean percentage changes (%ch.) in the total length of *L. fuscus* and *C. trinitatis* tadpoles kept two days under various treatments (see text for details). ANOVA performed on arcsin-transformed percentage length changes for each species separately, *** P<0.001. *Post-hoc* comparisons were A with B, C and D; C with D: superscripts which differ indicate significant differences between treatments (P<0.05).

Measurement (means±SD)	A Controls from foam nests		B Out of foam on damp tissue		C Water with no food		D Water with food		Growth after 1 day	Growth after 2 days
	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days	*F*-ratio	*F*-ratio
Body length-snout to hind limb base (mm)	3.9±0.1^{a}	4.1±0.2^{a}	3.5±0.2^{b}	3.6±0.2^{b}	4.2±0.3^{a}	4.8±0.3^{c}	4.6±0.3^{c}	6.0±0.6^{d}	$F_{3,30}$=28.1***	$F_{3,35}$=88.0***
	t =1.81, NS		t =0.51, NS		t =5.06, ***		t =7.60, ***			
Total wet weight (mg)	6.2±0.1^{a}	7.1±1.2^{a}	4.5±0.4^{a}	5.3±1.2^{a}	7.5±1.1^{a,c}	10.7±2.1^{a}	11.3±2.5^{b}	24.9±6.1^{b}	$F_{3,26}$=26.0***	$F_{3,33}$=64.3***
	t =1.01, NS		t = 1.49, NS		t =4.21, ***		t = 6.81, ***			
Total dry weight(mg)	1.6±0.2^{a}	1.2±0.1^{a}	1.1±0.2^{b}	0.6±0.2^{b}	1.5±0.4^{a}	1.1±0.4^{a}	1.6±0.4^{a}	2.8±0.9^{c}	$F_{3,27}$ =3.5NS	$F_{3,33}$=31.2***
	t = 3.59, **		t =3.91, **		t = 2.02, NS		t = 3.80, ***			
n	3	8	8	11	11	8	12	12		
Stage (Gosner)	27	28	27,27+	27+,28	27,27+,28	28	27, 27+,28	28,28+		

TABLE 2. Growth of tadpoles removed from foam on reaching stage 25 (day 4). Results pooled from 3 clutches. *t*-test results given for 1-2 day comparisons. *F*-ratios compare treatments using ANOVA. Probability values: ***=<0.001; **=<0.01; NS=>0.05. A was compared with B, C, and D; B was compared with C; and C was compared with D: superscripts which differ indicate significant differences between these groups (P<0.05).

Measurement (means ± SD)	A Controls from foam nests	B Water with no food		C Water with food		*F*-ratio	*t*-test
	1 day	1 day	2 days	1 day	2 days	(1 day)	(2 days)
Body length-snout to hind limb base (mm)	4.1±0.2^{b}	4.6±0.4^{b}	5.3±0.6	5.3±0.3^{c}	7.7±0.7	$F_{2,19}$=28.6***	t = 7.1***
		t = 2.78,*		t = 8.80,***			
Total wet weight (mg)	6.8±0.8^{a}	8.7±2.1^{b}	13.8±3.6	15.2±1.4^{c}	55.1±11.6	$F_{2,18}$=51.8***	t = 9.0***
		t = 3.40,**		t = 8.99,***			
Total dry weight (mg)	1.2±0.1^{a}	1.2±0.3^{a}	1.6±0.5	1.4±0.3^{a}	5.9±1.5	$F_{2,18}$=2.2NS	t = 7.1***
		t = 2.05,NS		t = 7.59,***			
n	6	8	7	7	8		
Stage (Gosner)	28	28,28+	28,28+	28	29,30,30+		

TABLE 3. Growth of tadpoles removed from foam on reaching stage 28 (day 6). Results pooled from 2 clutches. *t*-test results given for 1-2 day and 2-2 day comparisons. ANOVA results given for 1 day comparisons. Probability values: *** = <0.001; ** = <0.01; * = <0.05; NS = >0.05. Superscripts which differ indicate significant differences in 1-day comparisons (P<0.05).

Measurement (means±SD)	A Controls from foam nests (1 day)	B Out of foam on damp tissue 1 day	B 2 days	C Water with no food 1 day	C 2 days	D Water with food 1 day	D 2 days	*F*-ratio 1 day	*F*-ratio 2 days
Body length snout to hind limb base (mm)	3.7±0.04[a]	3.7±0.1[a]	3.8±0.1[a]	4.1±0.1[b]	4.2±0.1[b]	4.8±0.0[c]	5.8±0.1[c]	$F_{3,14}$=180.4***	$F_{2,9}$=414.7***
		t = 0.88, NS		t = 0.99, NS		t = 21.41, ***			
Total wet weight (mg)	7.0±0.4[a]	7.6±0.4[a]	8.2±0.7[a]	10.1±0.6[b]	11.2±1.0[a]	22.5±1.5[c]	40.5±4.2[b]	$F_{3,14}$=337.6***	$F_{2,9}$=200.0***
		t =1.43, NS		t = 1.90, NS		t = 8.09, ***			
Mitotic counts									
-brain	0.8±0.4[a]	0.6±0.3[a]	-	4.6±1.3[b]	4.0±1.1	21.2±2.6[c]	28.4 (1)	$F_{3,11}$=195.7***	-
-limb epidermis	0	0	-	0	0	12.4±1.0	8.6 (1)	$F_{3,11}$=30.0***	-
-limb mesenchyme	0.1±0.2[a]	0[a]	-	1.2±1.1[a]	5.4±2.2	63.6±14.2[b]	55.6 (1)		
n	6	4	4	4	4	4	4		
Stage (Gosner)	28	28	28	28	28	28	29		

TABLE 4. Growth of tadpoles removed from foam after about 6 days of developmental arrest (12 days after deposition). Results of a single clutch. *t*-test results given for 1-2 day comparisons. ANOVA compares treatments on 1 and 2 days, respectively: ***P <0.001. A was compared with B, C, and D; B was compared with C; and C was compared with D: superscripts which differ indicate significant differences between the groups (P<0.05).

three possible ways (water with or without food; on damp tissue paper as individuals out of foam) for one or two days. Measurements made on these tadpoles are shown in Tables 2, 3 and 4.

When stage 25 (start of foam-making, Table 2) tadpoles were transferred to water, they grew in length whether or not food was present. However, in the absence of food, there was no increase in dry weight, suggesting that the length increase was largely due to tissue hydration, a conclusion supported by the wet weight results. In fed tadpoles, dry weight only increased significantly over controls after two days, suggesting that stage 25 tadpoles were not yet able to utilize the external food supply. Fed tadpoles were larger than controls after two days, but not developmentally more advanced, showing that over this period, tadpoles in foam were developing at more or less the maximal rate. Tadpoles kept out of foam, on damp tissue, decreased significantly by all three measurements compared with controls in foam.

When tadpoles at the start of developmental arrest were transferred to water with food (Table 3), they grew significantly in length and wet weight, but not in dry weight compared to controls after one day; after two days, they had grown considerably more and by all measures had advanced morphologically well beyond the arrest stage (from stage 29 to 30 and beyond). These results show that on entering stage 28, tadpoles were capable of using food for growth and development immediately, and had no requirement to enter developmental arrest. When transferred to water with no food significant growth in length occurred but not in wet or dry weight, but less than with food, and the difference between one and two days was considerably less than in the case of fed tadpoles. With no food, the increase in dry weight between one and two days was not significant and there was no consistent advance in morphological development.

After six days of developmental arrest, tadpoles transferred to water with or without food grew similarly (Table 4) to those transferred at the start of the arrest period (Table 3). Weight measurements for this series are not directly comparable to those in Tables 2 and 3 because of the different procedure used for measurement, before preparing these tadpoles for histology. The mitotic count figures show that cell proliferation increased even in water without food after one day, then remained around that level, while in fed tadpoles, proliferation increased much more markedly. Some tadpoles were fixed after only half a day (results not shown) and in these, increased mitotic activity was already evident in fed tadpoles, but not in unfed ones. Tadpoles removed from foam and placed individually on damp tissue paper did not grow nor did they show any signs of escape from developmental arrest. However, unlike those treated in this way at stage 25 (Table 2), they maintained their length and weight, rather than decreasing.

DISCUSSION

The experiments described here are an attempt to clarify and test a suggestion by Pisano & Del Rio (1968) and a subsidiary hypothesis of Wassersug (1986) that tadpoles of the '*fuscus*' group that stay in the foam-nest are inhibited from developing beyond a certain point by factors present in the foam. In Pisano & Del Rio's experiments, growth was measured from the tail-bud stage (stage 17) in tadpoles either kept in foam or transferred to water without food. Tadpoles transferred to water were longer after only one day than those kept in foam: both groups of tadpoles then grew at very similar rates until day 4, when growth essentially stopped (with tadpoles in water 1-2 mm longer than tadpoles in foam) though in water, further significant growth occurred at around day 11. Histological examination of the two groups revealed no differences in chromatophore distribution or gut morphogenesis, but the yolky material in gut epithelial cells was used up more quickly in the tadpoles in water.

Pisano & Del Rio (1968) argued that in foam, there was an inhibition of *growth*, but not of differentiation or morphogenesis, and that this was demonstrated by the rapid increase in length as soon as larvae were transferred to water. However, since even in their own experiments, growth *rates* in the two groups were essentially the same for the next few days, it is difficult to argue for the existence of an inhibitor at this time and more reasonable to suggest that the initial size increase on transfer to water was the result of tissue hydration, a conclusion supported by the results presented here. In a separate paper, I have shown (Downie, 1994) that in *L. fuscus* tadpoles development is continuous in nest foam until stage 28, when growth and morphological development essentially cease, and not simply growth alone. It is particularly interesting that development ceases at this stage, since this is soon after the onset of foam-making by the tadpoles themselves (Downie 1984, 1989). As part of a general hypothesis on developmental inhibition, Wassersug (1986) suggested that developmental arrest in 'fuscus' type tadpoles may be controlled by an inhibitor, possibly prostaglandin E_2, secreted into the foam mucus by the tadpoles themselves. Mobbs, King & Wassersug (1988) found that prostaglandin E_2 did not inhibit thyroid hormone-induced tadpole tail metamorphic changes *in vitro* but left open the possibility of other inhibitory factors being present in oral mucus.

The results of my experiments make oral mucus inhibition very unlikely. A growth and development inhibitor might be expected to be effective over a range of stages as is the case for the antagonistic hormones regulating metamorphosis (Delidow, 1989), but the results showed that tadpole foam did not inhibit development of tadpoles before stage 28. Later-stage tadpoles out of water declined in length in any case, and by no more in foam than out of foam. Finally, developmentally-arrested stage 28 tadpoles isolated as individuals on damp tissue where they did not make foam did not show a growth and development spurt: they remained at essentially the same size and stage as when they were in the foam.

This conclusion does not, of course, mean that the foam made by the tadpoles has no role in their life in the nest. Preliminary experiments (Downie, unpublished) show that tadpoles in foam are able to survive several weeks, whereas isolated tadpoles last out of water for only a few days: the foam clearly has some function, yet to be clarified, in their survival. Similarly, the original nest foam clearly has some role in supporting early development, since eggs isolated on to damp tissue at pre-hatching stages generally failed to develop, whereas those in water or in foam developed well.

If the foam does not contain a development inhibitor how may developmental arrest be controlled? It appears that arrest occurs automatically at a particular stage of development, so long as the tadpoles remain in the foam nest, rather than in conditions suitable for further development. Development proceeds continuously till stage 28 in all conditions, then stops if tadpoles remain out of water, but continues if they are in water. There is no evidence for arrest being obligate. Arrest is not simply the result of a lack of food, since the gut endoderm still contains abundant yolk when arrest begins (Downie, 1994). A mechanism for automatic, stage-specific arrest can only be speculative at present. Arrest before metamorphosis is common in invertebrate larvae, and appears to be genetically regulated (Berking, 1991). The results obtained so far do not entirely answer the question of whether the presence of an external food supply is essential for developmental arrest to be released, or whether swimming in water, even in the absence of food is an adequate stimulus. Arrested tadpoles transferred to water alone generally increased in wet weight and body length, at least after two days, but dry weight changes, where these were measured, were not significant. The most interesting result in this context was the stimulation of mitotic activity in six day arrested tadpoles after only one day in food-free water. It is possible that in water, the gut yolk reserves are mobilized to allow some degree of growth and development, a suggestion supported by Pisano & Del Rio's (1968) histological results.

Another possible mechanism for developmental arrest requires some discussion here. It is well known that tadpoles are able to inhibit one another's growth, though Petranka's (1989) results suggest this may be commoner under laboratory conditions than in the field. Richards (1958, 1962) suggested that this kind of inhibition was mediated by algal infections passed via the faeces from tadpole to tadpole. Steinwascher (1979) suggested that yeasts of the genus *Candida* were the more likely infective agent and that inhibition was more severe on small tadpoles than on larger ones. Beebee (1991) provides strong evidence that the micro-

organisms concerned are unpigmented algae of the genus *Prototheca*. As I have reported (Downie, 1994) *L. fuscus* tadpoles in foam contain numerous small round cells in their intestines: these accord very well with the descriptions of the infective agents given by Richards, Steinwascher and Beebee. It is possible that *L. fuscus* developmental arrest could be mediated by *Candida/ Prototheca*, since the onset of arrest follows 1-2 days after foam-making begins, related to the onset of buccal activity and therefore feeding. Tadpoles may take up the micro-organisms from the soil or foam, and with the low rate of gut through-put likely in tadpoles out of water, these could multiply very rapidly. The tadpoles at this stage are very small, a factor suggested by Steinwascher (1979) to accentuate growth inhibition. It should be possible to test this explanation by rearing *L. fuscus* in micro-organism-free conditions. It is made somewhat unlikely by the rather precise, stage-specific nature of the developmental arrest process.

Another question, arising from the results presented here, is whether the ability to emerge from developmental arrest remains unchanged as tadpoles stay for prolonged periods in foam. The results suggest that the initial growth response once tadpoles enter water is faster for newly-arrested tadpoles than for those arrested for six days. In the context of foam nests as a survival strategy, this question is worth further investigation.

An unexpected result from the experiments reported here was that pre-hatching eggs added to heaps of foam-making tadpoles were generally eaten. There are several possible explanations for this, worthy of further investigation. Early stage embryos in the nest at this stage would normally be dead eggs, a possible source of infection - removal of them by consumption would be a matter of hygiene. In addition, their high yolk content would be a source of nutrition useful in maintaining tadpoles in the nest. Another possibility is that the added eggs are recognized as foreign and eliminated as a competition device.

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COMPARATIVE STUDY OF THE DORSAL PATTERN IN *SALAMANDRA SALAMANDRA BEJARAE* (WOLTERSTORFF, 1934) AND *S. S. ALMANZORIS* (MÜLLER & HELLMICH, 1935)

JAIME BOSCH[1] AND INMACULADA LÓPEZ-BUEIS[2]

[1]*Departamento de Ecología Evolutiva, Museo National de Ciencias Naturales, CSIC, José Gutiérrez Abascal, 2, 28006 Madrid, Spain*

[2]*Departamento de Biología Animal I, Facultad de CC, Biológicas, Universidad Complutense, 28040 Madrid, Spain*

The dorsal pattern of six populations of *Salamandra salamandra* was studied. Several quantitative characters of the dorsal spots (number, area, perimeter, axes length, co-ordinates of the centre of gravity, etc.) were analysed using computer assisted technology. The study of these characters results in unexpected clustering of the populations. The population of Macizo de Peñalara, which is considered within *S. s. bejarae* seems to be closely related to the *S. s. almanzoris* populations.

INTRODUCTION

Salamandra salamandra is a remarkably polymorphic species in terms of the dorsal pattern. Until now, in the Iberian Peninsula the presence of seven subspecies has been recognized, though not by all researchers (Barbadillo, 1987): *S. s. bejarae*, *S. s. almanzoris*, *S. s. gallaica*, *S. s. bernardezi*, *S. s. fastuosa*, *S. s. crespoi* and *S. s. terrestris*. The subspecies were described on the basis of both the position and the extension of the dorsal spots. *S. s. bejarae* is characterized by yellow or red spots scattered on the black dorsum. These spots are very variable in size, pattern and abundance. This subspecies is distributed throughout the Iberian Peninsula, except in the areas of distribution of the other subspecies (García-París, 1985). *S. s. almanzoris* shows a lower number of yellow spots, which are smaller and more dispersed. Its distribution is restricted to glacial lakes in Sierra de Gredos (García-París, 1985). *S. s. terrestris* shows yellow stripes arranged in two lines along the black back. It appears in France, although some authors are of the opinion that this subspecies is distributed across the Oriental Eastern Pyrineos Mountains and that it is present in some parts of Catalonia (Barbadillo, 1987). Eiselt (1958) studied the geographic variation of *S. salamandra* in Europe and the Middle East. He considered that the populations of *S. salamandra* of Montseny (Barcelona) belonged to *S. s. bejarae*.

The dorsal pattern of the family Salamandridae has been studied for many years in terms of their supposedly aposematic colouration (e.g. Howard & Brodie, 1973; Hensel & Brodie, 1976). In *S. salamandra* the dorsal pattern has been used in subspecific characterizations (Wolterstorff, 1934; Müller & Hellmich, 1935), and perhaps too frequently, authors have used only this character to assign local populations to a subspecies (Veith, 1992); Malkmus (1991) studied the geographic variation of the dorsal pattern in a large number of individuals of *S. s. gallaica* but he only analysed qualitative variables.

The dorsal pattern of *S. salamandra* has hardly been studied in a quantitative way. Degani (1986) only studied the number of yellow spots and determined the ratio between yellow and black areas. Here we determine if the affinities of several quantitative variables of the dorsal pattern of *S. s. bejarae* and *S. s. almanzoris* conform to the taxonomic classification traditionally accepted for the populations studied. Only quantitative variables were studied to eliminate any kind of subjectivity in the study of the dorsal pattern and to find if the pattern can be used to characterize these subspecies.

MATERIAL AND METHODS

The dorsal pattern of fifty adult individuals was analysed. The following populations were studied: Facinas (Cadiz) (n=9), Sierra Bermeja (Málaga) (n=10), Macizo de Peñalara (Sierra de Guadarrama, Madrid) (n=10), and Santa Fe del Montseny (Barcelona) (n=10), considered traditionally belonging to *S. s. bejarae* subspecies; Laguna Grande and Cinco Lagunas (Macizo Central de la Sierra de Gredos) (n=10, n=11, respectively) considered traditionally to belong to *S. s. almanzoris* . The individuals of Facinas, Montseny and Laguna Grande populations belong to the herpetological collection of the National Museum of Natural Sciences of Madrid (MNCN), and the sample from Sierra Bermeja belongs to a private collection. All preserved individuals were filmed with a video camera Sony V7AF 8 mm. Live animals were photographed in the field with the camera situated perpendicularly to the dorsal surface of the animal. A scale in cm was filmed with it. Films and photographs were digitized using a

Digi View Gold Digitalizator and a Commodore Amiga 500 computer. The images were analysed on a Macintosh IIsi using the Image 1.41 and Photoshop 2.0 software.

For each individual the following variables were measured: number of dorsal spots (ND), total yellow area (YA) and for each spot, the area (A) and perimeter (P). Total dorsal surface (yellow and black area), major axis length, minor axis length and co-ordinates of the centre of gravity of each spot were measured in order to calculate three new variables: the percentage of yellow area (%Y), axes ratio (M/m) and minimum distance across the centre of each dot (Dist). An arcsin linear transformation was applied to the variable percentage (ASIN%Y) and a square root transformation to the area variable (SQRA). The M/m variable gives us an idea of the shape of the spots.

To remove the effect of the size of the animal in the variables which were correlated with it, we used the residuals of the regression between each variable and the size of the animal (square root of the total surface). The analyses were performed using Statview II 1.03 program and the NTSYS statistical package (Rohlf, 1992). Classical statistical techniques were performed. A standardized matrix was used to calculate the "average taxonomic distance" between each pair of species and the clusters were generated through the application of an unweighted average (UPGMA) on the previously calculated matrix.

RESULTS

The average of the non-transformed variables studied for each population are shown in Table 1. The individuals of Sierra Bermeja were the largest, followed by the population of Facinas, while the smallest individuals were those collected in Cinco Lagunas and Laguna Grande. The number of dorsal spots is highest in Facinas (t=3.13; P<0.01), while the individuals of the Montseny population showed the lowest number of spots (t=4.36; P<0.001); spots in the latter population were larger than the ones of Facinas (t=3.08; P<0.01), the percentage of yellow area being very similar

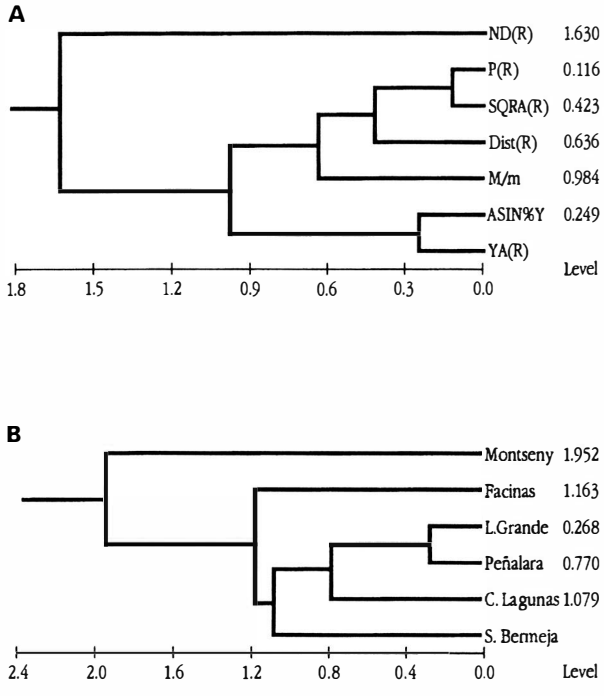

FIG. 1. A, Cluster of the variables analysed; B, Cluster of the populations studied.

(χ^2=1.02; NS). The M/m ratio is highest in the striped Montseny population (U'=82; P<0.01) and lowest for the dotted Cinco Lagunas population (U'=100; P<0.01). The individuals of Laguna Grande show the minimum percentage of yellow, being significantly lower than the other population of *S. s. almanzoris*, Cinco Lagunas (χ^2=20.82; P<0.001).

The ND, SQRA, P, SQRYA and Dist variables show a meaningful correlation with the size of the animal (F=10.2, P<0.01; F=29.6, P<0.001; F=22.8, P<0.001; F=136.3, P<0.001; F=27.0, P<0.001, respectively); while M/m and ASIN%Y show a non-significant correlation.

The average values of the residuals of the variables correlated with the size of the body and the average values of the rest of variables were standardized in order to calculate the cluster analysis. First we performed a cluster of the variables (Fig. 1A) to analyse the proxim-

Population	*n*	Total Surface (mm^2)	ND	A (mm^2)	P (mm)	M/m	Dist (mm)	%Y
Montseny	10	2795.8 (319.6)	22.1(4.4)	41.5(6.6)	20.0(2.3)	2.4(0.2)	8.5(1.0)	32.7(6.6)
Laguna Grande	10	2352.9 (363.5)	33.0(6.5)	18.1(3.5)	10.8(1.6)	1.9(0.2)	5.8(0.9)	25.2(4.9)
Cinco Lagunas	11	2258.3 (176.6)	34.8(4.5)	20.4(4.7)	11.1(2.1)	1.6(0.1)	5.9(0.7)	31.2(6.7)
Peñalara	10	3322.2 (821.8)	36.0(4.9)	24.8(6.3)	13.3(2.7)	1.9(0.1)	6.6(1.3)	27.5(8.5)
Facinas	9	4615.7(1379.4)	43.5(7.1)	33.3(4.8)	17.0(1.9)	2.0(0.2)	6.9(0.6)	32.5(4.5)
S. Bermeja	10	4924.8 (680.0)	33.0(7.6)	44.1(12.1)	19.6(4.1)	1.9(0.2)	9.5(1.7)	28.6(6.3)

TABLE 1. Mean and standard deviation of the variables analysed

ity among them. The variable ND seems to be far from the rest of variables. In a second group we find the two variables YA and ASIN%Y. The perimeter, SQRA and the minimum distance are together in another group. The SQRA and the perimeter are logically the most related variables. Considering the results of this cluster analysis another cluster was performed using the average values of the populations after removing the SA and P variables (Fig. 1B). The Montseny population appears far from the other populations, this is the expected result because of its different dorsal pattern. The rest of the populations are less differentiated and the most striking result is that the most closely related populations are Laguna Grande and Peñalara.

DISCUSSION

The quantitative analysis of the dorsal pattern seems to be more useful for defining the subspecies of *S. salamandra* than simple qualitative descriptions.

The population of Montseny is different from the other populations, with a striped dorsal pattern very similar to that of *S. s. terrestris*. This could be explained by assigning this population to *S. s. terrestris* , and not to *S. s. bejarae*, in contrast to Eiselt (1958).

The striking resemblance of the dorsal pattern in the populations of Peñalara and Laguna Grande can be interpreted in two different ways. It could be that the dorsal pattern in *S. salamandra* reflects different "ecomorphs". This hypothesis is supported by the fact that Peñalara and Laguna Grande are very similar habitats (high mountain).

On the other hand, it could be explained by the existence of high genetic proximity between both populations. This hypothesis is supported by genetic studies performed by Alcobendas, Dopazo & Alberch, (1993) where several Iberian Peninsula populations were analysed, and the idea of *S. s. almanzoris* occurring as relict populations was rejected.

A way of studying the validity of the dorsal pattern in the characterization of subspecies would be to check whether populations which are ecotypically different but geographically close show similar patterns.

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REPRODUCTIVE STRATEGY IN A MONTANE POPULATION OF THE LIZARD *LACERTA SCHREIBERI* (SAURIA: LACERTIDAE)

ADOLFO MARCO, VALENTIN PÉREZ-MELLADO[1] AND MARTIN J. GIL-COSTA

Department of Animal Biology, Universidad de Salamanca, 37071 Salamanca, Spain

[1] *Present address: Department of Environmental Sciences and Natural Resources, Section of Animal Biology, Universidad de Alicante, 03080 Alicante, Spain*

The mountain population of *Lacerta schreiberi* studied at the Sistema Central (Spain) showed a narrow and highly synchronized reproductive period, adjusted to short periods of annual activity, that are characteristic of these Iberian areas. Adult females reached sexual maturity at a minimum age of four years. Only one clutch of eggs is laid per year. The average clutch size of 14 eggs was positively correlated with female body size. Clutch weight was high (25 to 56% of net female weight), and incubation time was long (65 to 110 days). During incubation eggs reached three times their initial weight. We found a noticeable variability in some reproductive characteristics during the three years under study and among individual females. The greatest phenotypic plasticity was in the size of the eggs, incubation time, and the size of hatchlings, while clutch size showed a higher stability in its average values.

INTRODUCTION

Reproductive traits of several species of lizards can be considered unstable characteristics with important variation between populations (Ballinger, 1979, 1983; Braña & Bea, 1987; Dunham, 1978; Pianka, 1970; Tinkle & Ballinger, 1972), as well as within populations (Bauwens & Verheyen, 1987; Ferguson, Snell & Landwer, 1990; Pilorge, Xavier & Barbault, 1983). Such variability is due to annual variation of climatic factors and/or individual differences (fat reserves, body size, annual frequency of clutches), and suggest that some selective pressures are involved in producing adaptive phenotypes (Ferguson, Brown & Demarco, 1982).

The best strategy to study reproductive traits seems to be the long term tracking of marked individuals in populations of the temperate area. Such populations are subject to unpredictable annual variations of climate that promote differential selection of individual phenotypes (Braña, Bea & Arrayago, 1991; Frankenberg & Werner, 1992; Pilorge *et al.*, 1983; Shine, 1983).

In this work we present basic information about the reproductive characteristics of *Lacerta schreiberi*, an endemic lacertid lizard that inhabits humid areas of mountainous regions of the Iberian Peninsula (Salvador, 1984).

We studied clutch size variation, laying period, and appearance of hatchlings in natural conditions during three consecutive years in a mountain area where annual activity is reduced. We compare our data with those of two previous studies made in different areas of the northern Iberian Peninsula (Braña, 1983; Galán, 1986), as well as with scarce information which appeared in other works (Salvador, 1974, 1984; Barbadillo, 1987; Marco & Pérez-Mellado, 1989).

MATERIAL AND METHODS

The low probability of finding clutches of lacertid lizards in the field, especially in stony areas, precluded their study in fully natural conditions. For this reason we studied oviposition and incubation phenology under semi-natural conditions.

Hence, throughout the years 1990, 1991, and 1992 twenty-six gravid females were captured from a population of *Lacerta schreiberi* inhabiting an Holm oak forest of *Quercus pyrenaica*, at an altitude of 1250 m in the area of Dehesa de Candelario, Sistema Central (province of Salamanca, Spain, U.T.M. co-ordinates: 30TTK653691). Individuals were then housed in open-air terraria with open tops of 120 x 60 x 60 cm, situated at a distance of 3 km from the collecting site. Terrarium floors were covered with a 20 cm depth of natural soil from the collecting site and maintained under natural conditions of temperature, humidity and photoperiod. Each lizard was housed individually. We fed lizards with a mixed diet of *Tenebrio molitor* larvae and *Gryllus campestris*, providing water *ad libitum*.

Every three days we recorded the snout-vent length (SVL) (± 0.1 mm) and weight (± 0.02 g) of each female. The reproductive state of each female and its corresponding behaviour was observed at least twice daily from its capture to the first day of oviposition. After laying the last egg, each female was measured, weighed, and then released at the capture site. In all cases each clutch could be unequivocally assigned to an individual female so, the relative clutch mass (RCM) was estimated as the quotient between the weight of the clutch, taken immediately after laying, and female mass (excluding the clutch mass). We consider adjusted clutch sizes as the residual values of the regression analysis of clutch sizes on SVL of corresponding females.

Each clutch was placed in a plastic container (20 cm in diameter, 15 cm height) and covered with 5 cm of wet sand. The incubation was done in the same conditions as mentioned above for terraria (natural temperature and constant humidity). Eggs were measured (± 0.01 mm), weighed with an electronic balance (± 0.02 g) and their volume estimated as an ellipsoid. These measurements were repeated for each egg twice weekly until hatching. Embryonic growth rate was therefore estimated as the quotient between egg measurements (weight, volume, length and width) before hatching and after laying. Newborn lizards were marked, measured and weighed within 24 hr of hatching.

In addition, the reproductive phenology was observed in a study plot of approximately 2 Ha situated at the site of collection previously described, during 1989-1992. Seventy-one females were captured and marked by toe-clipping. For each female we recorded reproductive state (oviductal eggs, copulation marks and lateral skin folds), SVL, and weight. The age at maturity was known from the lizards marked in their first or second calendar years (Marco & Pérez-Mellado, 1990). Natural phenology observed during this study was similar to dates recorded from clutches developed under semi-natural conditions.

Climatological information was obtained from La Angostura Weather Station (National Meteorological Service), situated 1 km from the capture site, 3 km from the incubation site, and at a similar altitude to both localities.

Average annual rainfall in the period 1988-1991 was 1.015 litres/m². Average temperature for the same period was 10.96°C, with a minimum value of 4.30°C and a maximum of 18.02°C. Highest temperature recorded was 34°C, and lowest -3.2°C. Diel variation of temperatures ranged from 5.76°C to 21.14°C, with an annual average of 13.38°C.

Descriptive statistics included arithmetic mean ($\bar{x}$), standard deviation (SD) and 95 % confidence intervals (Sokal & Rohlf, 1969). Comparisons between years were made with ANOVA analyses of data previously checked for homoscedasticity (F_{max} test) and normality (David test, Martín-Andrés & Luna del Castillo, 1990). Comparisons between individuals were made with Pearson correlations, analyses of partial correlation and linear regression (Sokal & Rohlf, 1969; Martín-Andrés & Luna del Castillo, 1990).

RESULTS

Reproductive Phenology

Reproductive behaviour of females begins a few days after the onset of annual activity, that is, around the second week of April. Matings were observed during May and the first clutches of eggs were laid from the last week of May to the first half of June. First hatchlings appeared in the third week of August at 1990, in the second half of August at 1991 and in the first half of September at 1992, just before the end of the annual activity period.

It is interesting to point out the striking synchronization of matings as well as of the development of eggs. This observation was consistent, even in the course of years with cold and rainy springs (1990 and 1991). In fact, a great amount of phenological variability was due to annual differences (Table 1). The mating period was always less than 25 days, and every year, the first oviposition was made after the last mating of all females.

Reproductive Females, Clutches and Hatchlings

Minimum reproductive body size recorded for females in the population under study was 91 mm SVL (Table 2) with an estimated age of four or five calendar years (males were mature at three or four calendar years). All observed females above this body size showed reproductive behaviour.

Only one annual clutch with a high egg number (mean=13.7; 7-24 eggs) is confirmed for *Lacerta schreiberi* in the study. Also we observed a long incubation time between 65 and 110 days (average= 84 days). The average RCM was 37%, with a highly variable egg survival rate (62.5%, Table 2). All the non-viable eggs were fertilized and the largest part of those embryos died at the end of the incubation period.

One day before the laying of the first egg, the female starts intense digging activity using the forelegs. She constructs a narrow, deep and long gallery, only limited by terrarium size, that ends in a circular cavity of 4 to 7 cm in diameter. Eggs are deposited in a crowded mass, sometimes strongly stuck together. The cavity is closed with natural ground after laying.

Egg sizes at laying and a few days before hatching are shown in Table 3. We can see an important growth in weight and volume during the incubation period, attaining as much as three times the initial values (volume ratio, $\bar{x}$=2.78, SD=0.518; weight ratio $\bar{x}$=2.84, SD=0.342). The difference between the two growth ratios was not significant (t=0.517, P=0.61). Egg width (ratio: $\bar{x}$=1.42, SD=0.094) grows slightly more than its length (ratio: $\bar{x}$=1.36, SD=0.103), but this growth rate does not influence the final weight of hatchlings (Pearson correlation coefficient, weight ratio vs hatchling weight: r=0.140, P=0.514; volume ratio *vs* hatchling SVL: r=0.05, P=0.81).

Two hundred and sixteen out of 320 eggs hatched. The elastic shell of the egg is broken and only after some hours could the emergence of the head be observed. Morphometrics of newborn lizards are summarized in Table 4. Hatchlings are fully active on hatching. 32.5% of eggs stopped their development before hatching for unknown reasons. We did not find any statistical correlation between egg survival rate and recorded reproductive parameters (*vs* female SVL: r=0.095, P=0.65; *vs* RCM: r=0.15, P=0.47; *vs* egg mass: r=0.13, P=0.54; *vs* egg volume: r=0.16, P=0.46).

	YEARS			ANOVA TESTS		
	1990	1991	1992	*F*	d.f.	*p*
Female SVL	107.7	110.37	1.6.58	0.638	6,44	0.699
Clutch size	11.6	14.12	12.93	1.407	6,45	0.233
RCM	28.69	38.8	39.81	4.579	2,23	0.0212
Incub. days	68.75	74.00	96.21	35.745	2,21	<0.0001
Juv. no.	7.0	10.375	7.0	1.799	2,23	0.1880
Egg surv.	0.688	0.735	0.543	1.782	2,23	0.1908
Egg mass	0.658	0.677	0.763	7.158	2,23	0.0038
Egg volume	0.589	0.636	0.852	34.039	2,21	<0.0001
Hatch. SVL	30.96	31.54	32.06	8.598	2,213	0.0003
Hatch. weight	0.723	0.751	0.797	6.521	2,213	0.0018

TABLE 1. Annual variation of reproductive characteristics of *Lacerta schreiberi*. (Incub.=incubation; Juv.= juvenile; Surv.= survival; Hatch.= Hatchlings).

	n	$\bar{x}$	95% C.I.	SD	Min.	Max.
Female SVL (mm)	51	108.49	2.082	7.403	91	124
Female weight (g)	26	25.665	3.143	7.780	13.75	41.0
Clutch weight (g)	26	9.3708	1.1264	2.788	4.25	14.97
Clutch vol. (cm^3)	25	10.068	1.309	3.171	5.76	16.75
RCM (%)	26	37.361	3.284	8.129	25.85	56.06
Clutch size	52	13.7	0.95	3.41	7	24
Number offsprings	26	8.3	1.68	4.17	0	17
SR (%)	26	62.5	8.31	25.05	0	100
Incubation time	24	84.23	5.94	14.07	65	109.4

TABLE 2. Sizes of adult reproductive females of *Lacerta schreiberi* and general characteristics of clutches. *n*: sample size, $\bar{x}$: arithmetic mean; 95% C.I.: 95% confidence interval; SD: standard deviation; Min: minimum; Max: maximum; RCM: relative clutch mass; SR: survival rate of eggs (see text for more details).

	n	$\bar{x}$	95% C.I.	SD	Min.	Max.
I						
Weight (g)	320	0.716	0.010	0.0955	0.05	1.02
Length (mm)	320	13.80	0.129	1.172	11.5	17.3
Width (mm)	320	10.05	0.073	0.668	8.5	11.7
Volume (cm^3)	320	0.744	0.016	0.1474	0.44	1.13
II						
Weight (g)	293	1.977	0.040	0.3595	0.95	3.00
Lenght (mm)	293	18.51	0.20	1.740	13.7	26.5
Width (mm)	293	14.16	0.11	0.952	11.2	16.0
Volume (cm^3)	293	1.965	0.043	0.370	1.00	2.84

TABLE 3. Sizes of eggs from 26 clutches of *Lacerta schreiberi*. I: After laying; II: Before hatching. *n*: sample size; $\bar{x}$: arithmetic mean; 95% C.I.: 95% confidence interval; SD: standard deviation; Min: minimum; Max: maximum.

	n	$\bar{x}$	95% C.I.	SD	Min.	Max.
SVL(mm)	216	31.6	0.1959	1.46	27	35
Tail(mm)	216	45.7	0.601	4.48	14.0	55.5
Weight (g)	216	0.767	0.016	0,12	0.37	1.0

TABLE 4. Size of hatchlings of *Lacerta schreiberi*. *n*: sample size; $\bar{x}$: arithmetic mean; 95% C.I.: 95% confidence interval; SD: standard deviation; Min.: minimum, Max.: maximum.

Average weight of hatchlings was 38.8 % of egg weight nearest the time of hatching.

ANNUAL VARIATION

We did not detect statistical differences between years in the size of adult females, clutch size, survival rate of the eggs or reproductive success (Table 1). However, we found significant variability in the size of the eggs, incubation time, and hatchling characteristics (Table 1). It is interesting to point out that in years with longer incubation time we found higher weights and volumes of the eggs and, consequently, bigger newborn lizards.

Recorded temperatures during the incubation period were significantly different among years (Table 5). In 1992 temperatures were lower than in the remaining years of study and hence the longest incubation period was longer.

VARIATION BETWEEN INDIVIDUALS

There were strong correlations between clutch size, number of hatchlings, clutch weight and female body size (Fig. 1). However, egg and hatchling sizes were not related to female size (clutch size and RCM) (Table 6). RCM was independent of female weight (before laying,

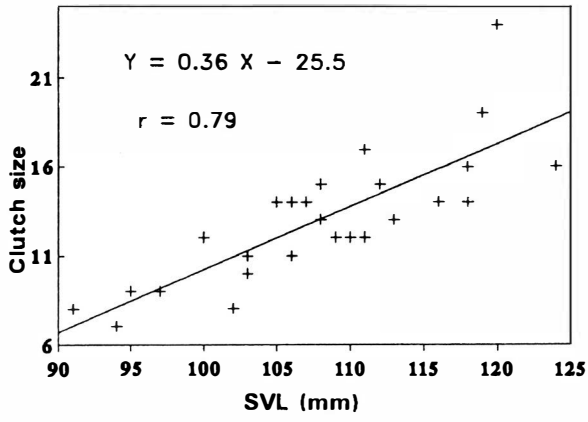

FIG. 1. Relationship between clutch size (egg numbers) and maternal SVL (mm) for *Lacerta schreiberi*.

Year	*n*	Incubation	T	Min.	Max.
1990	4	68.75 (1.31)	22.06 (0.20)	32.11 (0.20)	11.85 (0.14)
1991	8	74.00 (0.89)	19.59 (0.14)	27.54 (0.14)	12.25 (0.10)
1992	12	96.21 (2.71)	17.43 (0.21)	24.47 (0.26)	10.67 (0.16)
Total	24	84.23 (1.43)	18.92 (0.12)	26.77 (0.15)	11.40 (0.09)
$F_{2,21}$		35.745	100.80	179.50	33.979
P		<0.001	<0.001	<0.001	<0.001

TABLE 5. Annual variation of incubation time in *L. schreiberi* and ambient temperatures during these periods. Average and standard error. (*n*= sample size, T= average temperature, Max.=Maximum temperature, Min.= Minimum temperature, d.f.= degrees of freedom).

	Fem. SVL	Clutch size	RCM	Egg mass	Hat. SVL
Clutch size	0.862 (***)	1.000			
RCM	-0.58 (**)	0.608 (**)	1.000		
Egg mass	0.202 (NS)	-0.201 (NS)	0.347 (NS)	1.000	
Hatchling SVL	0.085 (NS)	-0.204 (NS)	0.054 (NS)	0.399 (NS)	1.000

TABLE 6. Coefficients of partial correlations between females lizards (*Lacerta schreiberi*) size and reproductive characteristics. At each correlation another three variables were controlled. Significance levels indicated thus: n.s.= $P>0.05$; **= $P<0.01$; ***= $P<0.0001$). (Fem.= female; Hat.= hatchling).

r=-0.217, P=0.287) and showed a partial negative correlation with female SVL (Table 6). Also, there was a positive correlation between average egg weight of each clutch just before laying and hatchlings SVL (stepwise model: coefficient=1.793, F=6.829, $P<0.01$).

DISCUSSION

The reproductive patterns found each year in an experimental plot accorded with the phenology of clutches kept in semi-natural conditions. Hence, we can discard strong artificial influences on laying dates and incubation times (Braña *et al.*, 1991; Cuéllar, 1984; Stamps, 1976) due to stress and subsequent inhibitory response of oviductal contractions (Jones & Guillette, 1982).

In natural conditions, all females are reproductively active throughout their adult life, with only one clutch per year. Reproductive periods are synchronized between females and strongly adjusted to the annual activity period. Hence, reproduction starts during an unfavourable period and newborn lizards appear a few days before the first frosts (Marco & Pérez-Mellado, 1989). Consequently, body size and weight of hatchlings can be regarded as adaptive factors (Nussbaum, 1981) influencing the survival of juveniles (Ferguson & Böhlen, 1978), that have only a short period to obtain fat reserves for wintering (Shine, 1983).

Relatively long incubation times found in *L. schreiberi* are similar to those of other green lizards, such as *Lacerta viridis* and *L. trilineata* (Nettmann & Rykena, 1984) or *L. agilis* (Bischoff, 1984). This long incubation precludes the development of a second clutch and suggests a synchronous pattern of annual reproduction. The main influence of temperature seems to be on the length of incubation.

The reproductive characteristics of the population under study showed some differences from data gathered by Braña (1983) and Galán (1986). In the coastal region of Galicia (north western Iberian Peninsula), females reach sexual maturity at a similar body size, likewise have only one clutch per year, but of larger size (average: 15.9) and smaller eggs (13.1 x 9.3 mm). Newborn lizards are also smaller (26.5 to 29.3 mm and 0.5 to 0.8 g) (Galán, 1986).

Hence, climatological constraints imposed by mountains and the shorter activity period (Marco & Pérez-Mellado, in prep.) compared to the coastal areas of Galicia, could be considered selective pressures for a higher investment in egg size (Nussbaum, 1981) at the expense of clutch size, maximizing the probability of hatchling survival (Shine, 1983). In the Asturias region, more favourable climatological conditions allow two clutches per year (Braña, 1983) each with fewer eggs (12.5) than in areas where only single clutches are produced.

The reproductive strategy of the lizards under study demands a concentration of reproductive effort during a short period of time. To maximize reproductive success, *L. schreiberi* possess a high relative clutch mass (average RCM: 37%), linked with a partial sit-and-wait foraging strategy and a cryptic antipredator escape tactic of the females (Marco & Pérez-Mellado, in prep.; Vitt & Price, 1982). Huey & Pianka (1981) proposed

that RCM is a consequence of foraging mode. In mountain zones, with a shorter activity period, an increase in survival of larger hatchlings (Ferguson & Bohlen, 1978) and a high mortality rate of the eggs, is probably related to an unstable environment (Ratterman & Ackerman, 1989). This may select for the evolution of higher RCM, and in some species, a sit-and-wait foraging strategy.

Furthermore, the synchronized oviposition precludes an inverse correlation between ovulation time and female size (Bauwens & Verheyen, 1985), promoting a delayed acquisition of sexual maturity (fourth or fifth calendar year) in comparison to males, and a very high relative clutch mass (maximum: 56%) in smaller females. Hence, during their lifetime, females tend to raise egg number (RCM dependent) instead of egg size (see Frankenberg & Werner, 1992). Egg size, is in fact, independent of female SVL and already maximized (see above).

The situation is different in the smaller lacertid lizard *Podarcis bocagei* (Galán, 1992), which inhabits unstable environments similar to those occupied by Schreiber's lizard. In such small oviparous species, to increase the small clutch size (4-5 eggs) by only one egg involves an increase of 15 to 20% in RCM, so it opts for a maximization of egg size (but see Frankenberg & Werner, 1992). However, in species with more than 10 eggs per clutch, as *Lacerta schreiberi*, the increase in reproductive effort to add one offspring is proportionally smaller. The relationship bewteen the number of annual clutches (*L. schreiberi*-one; *P. bocagei*-two, at least) and incubation period and body size (at the same climate) could be important to understand these differences between egg mass and clutch size.

We detected a noticeable increase in egg volume during the incubation, as was also found in other reptile species (Andrews & Sexton, 1981; Tracy, 1980; Deeming, 1989). This increase of egg volume is due to water input from external sources (Cagle, 1950; Legler, 1954; Whitaker, 1968), and allows eggs to be laid with a lower water content, lower volume and, consequently, higher clutch size. On the contrary, such hydric exchange could be unfeasible in arid regions, where the lack of soil water can be a direct mortality factor for embryos (Packard, Packard & Boardman, 1980). Perhaps, this could be an important constraint in the geographical distribution of *Lacerta schreiberi* (Marco & Pollo, 1989) and other species with a similar strategy.

Finally, our comparisons between years show clear differences in egg size, incubation period, egg survival rate and hatchling size. Hence, reproductive fitness can vary between years, with important effects on population dynamics and natural history of the species. This is, again, evidence for the phenotypic plasticity referred to by other workers (Ballinger, 1977; Dunham, 1981), and that must be studied to discover the physiological mechanisms and environmental variables involved (Marco & Pérez-Mellado, in prep.).

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DIEL VARIATION IN PREFERRED BODY TEMPERATURES OF THE MOORISH GECKO *TARENTOLA MAURITANICA* DURING SUMMER

MARTIN J. GIL[1] *, FATIMA GUERRERO[2] AND VALENTIN PÉREZ-MELLADO[3]

[1] *Institute of Nature Conservation, Kiewitdreef 3, B-3500 Hasselt, Belgium*

[2]*Departamento de Biología Animal, Sección Zoología, Universidad de Salamanca, Salamanca, Spain*

[3]*Departamento de Ciencias Ambientales, División de Biología Animal y Vegetal, Universidad de Alicante, Alicante, Spain*

**Address for reprints: Apartado de Correos 173, 37080 Salamanca, Spain*

We studied diel variation in preferred body temperatures (T_p) of adult and subadult geckos (*Tarentola mauritanica*) in a laboratory thermogradient. Overall T_p averaged 31.6°C and is significantly higher that the activity body temperatures recorded in the field. We did not detect differences in T_p between adult and subadult lizards. T_p varied according to a diel cycle. There was a gradual increase in T_p during the afternoon-early evening period, leading to peak values just before the beginning of the night-time activity period. During the subsequent hours, T_ps decrease again.

INTRODUCTION

Lizards are well known for their capacity to precisely regulate body temperature through the exploitation of environmental heat sources by means of behavioural adjustments (review in Huey, 1982). Nevertheless, some forms have only restricted access to heat during routine activities. Geckos provide a striking example. Most species of geckos restrict surface activity and foraging to the night, when ambient temperatures are low and opportunities for behavioural regulation are severely limited (Porter & Gates, 1969). Hence, it is not surprising that most nocturnal geckos exhibit lower and more variable activity body temperatures than sympatric diurnal lizards (Stebbins, 1961; Licht, Dawson, & Shoemaker, 1966*a*; Heatwole, 1976; Werner, 1976; Werner & Whittaker, 1978; Pianka & Huey, 1978; Avery, 1982; Pianka, 1986; Huey, Niewiarowski, Kaufmann & Herron, 1989). However, differences in activity body temperatures between diurnal and nocturnal lizards might reflect dissimilarities in thermal preferences, instead of being a direct consequence of differences in environmental constraints. In other words, the mere observation of lower and more variable body temperatures in nocturnal lizards does not provide information on the proximal mechanisms - environmental constraints and/or thermal preferences -that induce this difference (Huey, 1982). One way to overcome this problem is to assess thermal preferences, and use them as an independent yardstick for the evaluation of differences in activity body temperatures (Van Damme, Bauwens & Verheyen, 1986, 1987; Hertz, Huey & Stevenson, 1993).

The preferred (or selected [Pough & Gans, 1982]) body temperatures of ectotherms, measured in a laboratory thermogradient, indicate the range of body temperatures that lizards will maintain in the absence of abiotic and biotic restrictions (Licht *et al.*, 1966*a*; Van Damme *et al.*, 1986). They estimate the preference zone of body temperatures maintained by behavioural thermoregulation, and reflect a behavioural choice (Huey, 1982). However, the preferred temperature cannot be considered as a fixed characteristic for a given species, because it has been shown to be subject to considerable intraspecific variation (Huey, 1982).

Here we present results of a preliminary study of the preferred body temperatures of the gecko *Tarentola mauritanica*. This is a medium-sized gecko (adult snout-vent length: 47.4 - 75.1 mm) found over most of the Mediterranean areas of Europe. It is very often associated with human habitations, and restricts foraging to the night, although in some parts of its range individuals may be seen basking in sunshine during early morning or late afternoon. Our objectives were to (1) determine preferred body temperatures and critical thermal minimum and maximum temperatures; (2) examine differences in thermal preferences between age classes; and (3) explore the extent of diel variations in thermal preferences. In a forthcoming study, we will use this information to evaluate proximal causes of variations in activity body temperatures.

MATERIALS AND METHODS

Five adult and five subadult (= immature) geckos were caught during July 1992 near Candeleda (40°05' N - 05°10' W; prov. Avila; Spain; altitude = 400 m). Lizards were transported to the laboratory, where they were kept for one week in large terraria on a 15L : 9D natural photoperiod. Water and food (mealworms) was available *ad libitum*. Lizards were marked individually with colour codes on the body.

The five adult lizards were then transferred to a thermal gradient. This was a rectangular terrarium (100 x 50 x 50 cm) with a 250 W ceramic heat bulb suspended above one end, and a cooling system with ice in the other. Light was provided during the day by a fluorescent tube suspended above the centre of the terrarium.

The substratum consisted of a 4 cm layer of sand. Fifteen floor-tiles (15 x 15 cm) were placed at regular intervals over the total length of the terrarium, with one side tilted 5 cm above the surface. The tiles provided both shelter and opportunities for thermoregulation by thigmothermy. Surface temperature in the gradient ranged from 19.3 to 68.7 °C. No food was provided, but water was available in small dishes. Lizards were given two days to habituate to the experimental conditions. During the next two days, we recorded the body temperatures of the lizards during two five hour periods: 1630 - 2030 hr, which corresponds to the warmest period of the day in the field, and 2230 - 0230 hr, which is the period of maximal lizard activity in nature (Gil, 1992). Each individual lizard was taken out of the gradient at hourly intervals, and we measured its cloacal (=body) temperature, to the nearest 0.1° C, with a thermocouple connected to an electronic thermometer (Digitron). We only took measurements for lizards that were undisturbed prior to capture. This procedure was then repeated for the group of five subadult lizards.

As we repeatedly sampled body temperatures of each individual lizard, we analysed these data by a two-way ANOVA with repeated measurements. Some lizards were disturbed prior to capture, so that on each experimental day some hourly body temperature records were missing for some individuals. Although the ANOVA designs allow for missing data, we retained for analysis the average body temperature recorded during the two days for each individual, time period and hour as this is the ideal situation for this method. The ANOVA was designed with two "within" factors (period [day or night] and hour) and one "between" factor (age class [adult or subadult]). This allows testing of differences in preferred temperatures between age classes, periods and hours, and of the interaction effect between these factors. We also used the Greenhouse-Geisser epsilon (G-G) and Hunyh-Feldt epsilon (H-F) correction (Keppel, 1991) because the sphericity assumption will rarely be satisfied in a repeated measurement study as the correlation between responses close together in time will tend to be greater than the correlation between responses far apart in time.

RESULTS

Preferred temperatures (T_p) of *Tarentola mauritanica* are normally distributed (Kolmogorov-Smirnov test: $DN = 0.03$, $P = 0.99$), so parametric analyses are appropriate. Overall, T_p averages 31.56°C (SE = 0.17, $n = 151$); the interquartile range (the range including the central 50% of the temperature measurements, a non- parametric statistic of variance) equals 2.55°C. The preferred temperatures are considerably higher than the activity body temperatures measured in the field during the same period and study area ($\bar{x}$ = 24.8 °C, SE = 0.51, $n = 30$ [Gil, 1992]; $t = 14.1$, $P < 0.001$).

The results of the two-way ANOVA are summarized in Table 1. There are no significant differences between lizard age-classes, time periods (day or night), or hours. However, the interaction effect of period by hour is highly significant (Table 1). T_ps increase gradually during the course of the day period, whereas they decrease during the night (Fig. 1). During each of the two periods, significant hourly variation in T_p were detected (one-way ANOVA with repeated measurements; day: $F = 3.71$, df= 4, 36, $P < 0.02$; night: $F = 3.20$, df = 4, 36, $P < 0.05$).

Variance in preferred temperatures did not differ between the day and night periods, or among hours within each of the two periods (Bartlett's test for homogeneity of variances, $P > 0.05$ in all cases).

	df	SS	MS	*F*	*P*	G-G	H-F
Age-class	1	4.22	4.22	0.44	0.52		
Subject	8	76.31	9.53				
Period	1	3.74	3.74	1.46	0.26	0.26	0.26
Period*Age-class	1	0.85	0.85	0.33	0.57	0.57	0.57
Period*Subject	8	20.51	2.56				
Hour	4	3.71	0.29	0.95	0.44	0.40	0.43
Hour*Age-class	4	2.06	0.51	0.52	0.71	0.60	0.67
Hour*Subject	32	31.23	0.97				
Period*Hour	4	24.82	6.20	5.11	0.00	0.06	0.00
Period*Hour*Age-class	4	2.94	0.73	0.60	0.66	0.61	0.66
Period*Hour*Subject	32	38.84	1.21				

TABLE 1. Results of the two-way ANOVA with repeated measurements, analysing variation in preferred body temperatures as a function of Period (day or night), Hour, Age-class (adult or subadult), and their interactions. df = degree of freedom; SS = sum of squares; MS = mean square; G-G : Greenhouse-Geisser correction; H-F : Hunyh-Feldt correction.

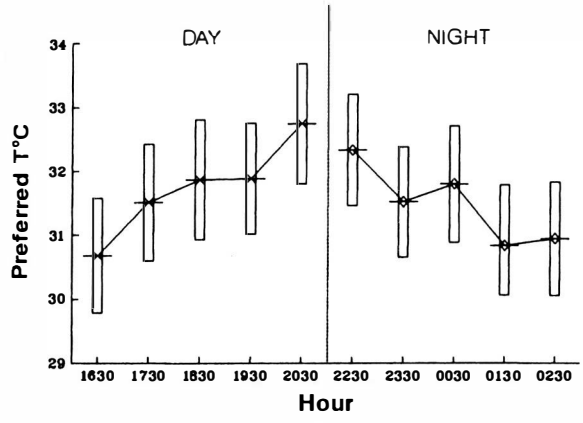

Fig. 1. Average (± 1 SE) preferred body temperatures for adult and subadult *T. mauritanica* at different hourly intervals during the day and night experimental periods.

DISCUSSION

Preferred body temperatures of *Tarentola mauritanica* recorded in a laboratory thermogradient averaged approximately 8 °C higher than the activity body temperatures recorded in nature. This result is consistent with that observed in many other geckos (Licht, Dawson, Shoemaker & Main, 1966*b*; Dial, 1978; Huey *et al.*, 1989). Given that T_ps are measured in a laboratory environment where abiotic constraints are absent (Licht *et al.*, 1966*a*; Van Damme *et al.*, 1986), we infer that environmental conditions in nature severely restrain the geckos' abilities to maintain body temperatures near the preferred range. We did not measure the thermal dependence of physiological or whole-animal performance traits, so that we cannot explore to what extent activity or preferred temperatures match the physiologically-optimal temperatures. In other nocturnal geckos, optimal temperatures for sprint speed are substantially higher than field body temperatures (mean difference for four species = 7.1 °C) , and preferred temperatures (mean difference for four species = 3.8 °C) (Huey *et al.*, 1989). Hence, these geckos generally forage at body temperatures that are suboptimal for sprinting (Huey *et al.*, 1989). These authors suggested that the thermal physiology of nocturnal geckos might be coadapted not to the low body temperatures maintained when foraging at night, but rather to the high body temperatures geckos may experience during the day, especially if cold diurnal retreats are unavailable (Heatwole, 1976; Dial, 1978; Huey, 1982).

Our results show significant diel variation in preferred body temperatures, even though ambient conditions in the thermogradient remained constant. Hence, lizards had the opportunity to maintain invariable body temperatures throughout the experimental period. We therefore interpret the observed diel fluctuation as an active shift in behavioural preferences. Diel cycles in T_ps have been observed in several other ectotherms (see review in Sievert and Hutchison, 1988). Together with observations of variation related to age, sex, reproductive condition, and season (e.g., Patterson & Davies, 1978; Beuchat, 1986; Van Damme *et al.* 1986; Daut & Andrews, 1993), they indicate that preferred temperatures cannot be considered as a fixed trait (Huey, 1982), but that they are subject to considerable intraspecific variation.

Preferred body temperatures of *Tarentola mauritanica* exhibit a gradual increase of approximately 2 °C during the final hours of the day, followed by an equivalent decline during the first five hours of the night. Consistent with the adoption of the highest T_ps during the late afternoon are field observations of overt basking behaviour at that time of the day (Martinez-Rica, 1974).

Two hypotheses may account for the increase in T_p during the final part of the day. First, a preference for relatively low body temperatures during the hot afternoon hours, may induce lizards to choose moderately cool retreat sites, thereby reducing the risk of overheating. Second, the maintenance of fairly high preferred temperatures during the late afternoon, just before the onset of surface activity in the field (Gil, 1992), can be considered as a mechanism to prolong the duration of night-time activity. Due to a scarcity of external heat sources, nocturnal geckos probably have limited opportunities to behaviourally control their body temperatures. Hence, body temperatures of active geckos will decline gradually during the course of the night, until they reach a lower threshold which induces lizards to cease surface activity (Bustard, 1967). At a given cooling rate, the duration of activity should therefore be a function of the body temperature maintained at the initiation of activity, with higher initial temperatures being associated with longer activity periods.

During the night, preferred temperatures are highest in the first few hours after sunset, coinciding with the period of maximum activity in the field (Gil, 1992) when environmental temperatures are still high (Bustard, 1967; Pianka & Pianka, 1976). The availability of external heat sources, and hence opportunities to raise body temperatures, probably decrease during the night. The observed decline of preferred body temperatures during the night might therefore be considered as a mechanism that tends to reduce the time spent in thermoregulatory behaviours.

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SHORT NOTES

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OBSERVATIONS ON THE SWIMMING OF THE PACIFIC RIDLEY, *LEPIDOCHELYS OLIVACEA* (ESCHSCHOLTZ, 1829): COMPARISONS WITH OTHER SEA TURTLES

JOHN DAVENPORT AND GAYLE A. PEARSON

University Marine Biological Station, Millport, Isle of Cumbrae, Scotland KA28 0EG, UK

One of the authors has previously described swimming mechanisms in three species of sea turtles; the green turtle *Chelonia mydas* L. (Davenport, Munks & Oxford, 1984), the loggerhead turtle *Caretta caretta* L. (Davenport & Clough, 1985, 1986), and the leatherback turtle *Dermochelys coriacea* (Vandelli) (Davenport, 1987). Living sea turtles have several common features that separate them from other chelonians (e.g. hypertrophied forelimbs, large pectoral muscles, streamlined shape, poor terrestrial locomotion, large egg clutches). These similarities mask marked ontogenetic and interspecific differences in life style (see Pritchard, 1979; IUCN, 1982 for general reviews). Leatherbacks are specialized oceanic wanderers that eat gelatinous prey, while herbivorous green turtles migrate great distances. The other sea turtles, the hawksbills (*Eretmochelys imbricata* L.), flatbacks (*Chelonia depressa* Garman), loggerheads and ridleys (*Lepidochelys olivacea* [Eschscholtz] and *Ledidochelys kempii* [Garman]) are more coastal and omnivorous.

Hatchling and small juvenile turtles appear to live in the open oceans, drifting with currents, particularly in weedlines, where they may subsist on food very different from the adult diet (e.g. Caldwell, 1968; Smith, 1968; Carr & Meylan, 1980; Stoneburner, Richardson & Williamson, 1982), although it has to be said that virtually all information is for green and loggerhead youngsters; young ridleys, hawksbills and leatherbacks have generally not been observed at sea after swimming offshore in a 'swimming frenzy'.

There are also differences in the diving abilities of young turtles. Hatchling green and loggerhead turtles (particularly the latter) dive with difficulty (Hildebrand & Hatsel, 1927; Milsom, 1975; Davenport *et al.*, 1984), yet neonate leatherbacks dive easily (Davenport, 1987).

Although interest in the locomotion of sea turtles initially centred upon the simultaneous beating of the forelimbs (Carr, 1952; Gray, 1953; Walker, 1971; Blake, 1981; Davenport *et al.*, 1984), later studies have revealed that swimming can be more varied, particularly at low speeds. Davenport & Clough (1986) showed that hatchling loggerheads employ a simultaneous double hindlimb kick for 95% of their swimming; they progressively switch to an alternate-leg forelimb kick as they grow beyond 400 g. Synchronous forelimb beating was seen much more rarely than in green turtles of similar size. In contrast, Davenport (1987) demonstrated that hatchling leatherbacks always swam with a synchronous forelimb beat.

The note reported here stemmed from an opportunity to study and film juvenile Pacific ridley turtles. It is supplemented by some qualitative observations made upon growing *Chelonia mydas*. Pacific ridleys, although widespread in the Pacific, Indian and Atlantic oceans (Pritchard, 1979; IUCN, 1982) are amongst the least studied of all sea turtles. With the Kemps ridley *Lepidochelys kempii* and the loggerhead *Caretta caretta*, they have been said to form a natural group (sometimes described as the subfamily Carettini - see Pritchard, 1979 for discussion). The jaws of the olive ridley are weaker than those of the loggerhead or Kemp's ridley (Pritchard, 1979) and it appears that adults forage for crustaceans in tropical neritic waters of considerable depth; they have been caught in shrimp trawls at 80-110 m, though they are also known to eat surface material such as jellyfish and fish eggs (Fritts, 1981; Mortimer, 1982). Pritchard (1979) reports that young Pacific ridleys have almost never been caught; only hatchlings or subadults have been seen in the wild. This suggests that the young live offshore, probably for several months or years.

The Pacific ridleys investigated (*ca.* 300-600 g body weight) had been held in large open-air tanks (1.5 m deep) for about 8 months at a head start facility at the Muka Head marine biological station of the Universiti Sains Malaysia, Penang, Malaysia. They had been reared from locally laid eggs and were fed on trash fish. Their tanks were continuously supplied with water pumped directly from the sea (35°/$_{oo}$; *ca.* 30°C).

The green turtles investigated were sent as hatchlings from the Lara Reserve, Cyprus, to the School of Ocean Sciences, University College of North Wales, where they were held in tanks of sea water (34°/$_{oo}$; 25°C) for one year, primarily for nutritional studies already reported (Davenport & Scott, 1993*a*, 1993*b*).

Two Pacific ridleys (347 g, 140 mm carapace length; 561 g, 247 mm carapace length) were filmed in a large glass tank (2 x 1 x 0.5 m) with a Panasonic F10 colour videocamera equipped with a high speed (0.001s) shutter. The tank had a 1 cm grid inked onto the rear surface; the turtles were filmed from a distance of 5 m to avoid parallax problems. Film was analysed by freeze-frame and playback through a Panasonic AG 6200 videorecorder and monitor, coupled with drawings made by placing acetate sheets over the monitor screen. Filming of the ridleys in the laboratory was supplemented by visual observations of ridleys and green turtles in their holding tanks.

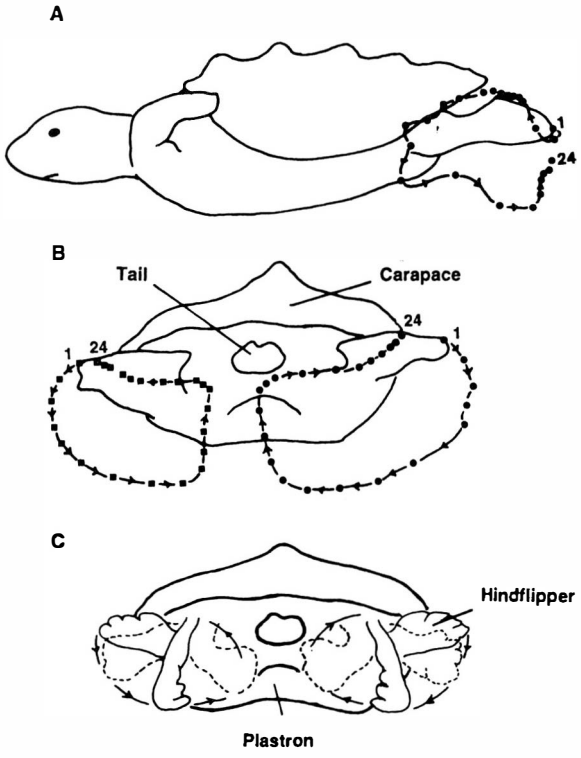

Fig. 1. Forward swimming by synchronized movements of the hindlimbs. Closed circles represent positions of fixed points on hindflippers in successive videofields (0.04 s apart). Numerals represent numbers of first and last fields. A. From side. Note that flipper moves fastest on the backwardly-directed effective stroke. Note also that the foreflippers are motionless. B. View of hindflipper action from behind. Note that effective stroke involves fast medially-directed movement of the hindflipper. C. Appearance of hindflipper at various stages of limbcycle. Note that hindflipper is held in scoop-like fashion at fastest part of effective stroke.

Ridleys were all ($n = 20$) positively buoyant, and had clear difficulty in remaining underwater; when they dived to feed on the bottom of their holding tanks they had to scull with their foreflippers to stay down, and fed with a tail-up attitude. The filmed animals were both very buoyant; if they swam downwards and ceased swimming they quickly bobbed up. The resting posture was very high in the water, with much of the shell protruding above the water line.

Hatchling green turtles had some difficulty in diving at first, but all of the animals studied ($n = 12$) were able to dive and exhibit neutral or negative buoyancy by the time they were 100-150 g.

When undisturbed, the ridleys either floated at the water surface, or swam slowly forwards (< 0.5 body lengths s^{-1}, with a synchronized sculling movement of the hindlimbs only; Fig. 1). The hindlimbs were extended and feathered during the recovery stroke, but curved together like scoops on the effective stroke; the hindlimbs of *Lepidochelys* are considerably larger and more flexible than green turtles of similar size. When swimming in this fashion, the foreflippers were held edge-on to the direction of movement, but were held out laterally and not tucked onto the top of the shell. If the turtle changed direction the hindflipper movements became asynchronous, or, if the animal was turning without forward movement, only one of the hindflippers would be moved. The turtles often swam slowly backwards (< 0.2 body lengths s^{-1}), both in their holding tanks and when filmed, sometimes for considerable distances. To do so they normally swam by approximately synchronized movements of the hindlimbs, though during manœuvres or short bursts of fast backward swimming, alternate-limb action was employed. When swimming slowly backwards with synchronized hindlimb action, the forelimbs were held extended sideways, but when swimming backwards with an alternate-leg action the foreflipper tips were stretched forwards, in front of the snout.

When the ridleys were disturbed by the approach of an observer, they initially responded by swimming with all four limbs in dog-paddle fashion with the limb action synchronized diagonally, the basic cryptodiran pattern (Zug, 1971) also seen in young green and loggerhead turtles (Davenport *et al.*, 1984: Davenport & Clough, 1986; Wyneken, 1988). Only when the turtles were handled did they respond by fast swimming involving synchronized forelimb beating. As with green and loggerhead turtles there was no sign that ridleys beat the forelimb tips in the figure-of-eight fashion claimed for the Family Cheloniidae by Walker (1971). During the upstroke and downstroke of the forelimbs the blade of the foreflipper was held in similar fashion to that of the green turtle (Davenport *et al.*, 1984), so propulsion during vigorous, synchronized foreflipper beating is based partially upon the generation of lift on both up and downstrokes, whereas hindlimb propulsion is purely drag-based (see Blake, 1981; Davenport *et al.*, 1984 for discussion).

All forward swimming in the young green turtles was by synchronized foreflipper beating, or by dog-paddling (as described by Davenport *et al.*, 1984). Only one extra mode of swimming was seen, and then not until the animals had reached a weight of about 500 g. Occasionally, *Chelonia* of this size or larger would swim backwards using an alternate hindlimb action, and with the foreflippers extended so far forwards that they touched in front of the snout, so that the head was completely hidden from the sides.

The swimming and buoyancy of young Pacific ridleys most closely resembles that of loggerheads amongst the living sea turtles so far studied. Like loggerheads they remain positively buoyant and incapable of sustained or deep diving for a long period after hatching. They also propel themselves most of the time by action of the hindlimbs, rather than the foreflippers. However, there are some noticeable differences. Young loggerheads swimming by synchronized hindlimb ac-

Swimming mode	*D. coriacea*	*C. mydas*	*C. caretta*	*L. olivacea*	*L. kempi*	*E. imbricata*
Forwards	+	+	+	+	+	+
Backwards	-	+	+	+	+	+
Foreflipper propulsion	+	+	+	+	+	+
Dogpaddle	-	+	+	+	?	?
Hindflipper forward propulsion						
(a) synch.	-	+	+	+	?	?
(b) alternate	-	-	+	+	?	?
Hindflipper backward propulsion						
(a) synch.	-	-	-	+	?	?
(b) alternate	-	+	+	+	?	?
Dominant part of foreflipper stroke*	up	down	down	down	?	?
Dominant hatchling slow swimming stroke**	foreflipper beat	dog-paddle	hindlimb action (synch.)	hindlimb action (synch.)	hindlimb action (synch.)	?

TABLE 1. Summary of known swimming mechanisms of sea turtles. Key: + = observed/reported; - = not observed/reported: synch. = synchronized; * leatherbacks beat foreflippers downwards and forwards, upwards and backwards; cheloniids beat them downwards and backwards, upwards and forwards: ** observed long after 'swimming frenzy' is over. Sources: this study; Walker (1971); Davenport *et al.*(1984); Davenport & Clough (1986); Davenport (1987); Wyneken (1988); Renous & Bels (1993).

tion fold the forelimbs against the carapace. Davenport & Clough (1986) suggested that such folding would reduce drag and perhaps protect the forelimbs against nibbling by fish. The ridleys did not show such folding. The ridleys also appeared to use synchronized foreflipper propulsion even less than loggerheads, their standard 'escape' response being to use dog-paddling rather than foreflipper beating. Generally they appear to have a low-energy lifestyle, compatible with drifting for long periods at the sea surface.

Wyneken & Salmon (1992) have recently reported on activity levels in hatchling turtles (expressed in terms of the proportion of time spent swimming during and after the swimming frenzy) and report activity levels in the order leatherback > green > loggerhead (though differences between green and loggerheads were minor). There is no doubt that leatherbacks are the most pelagic of living sea turtles, having (relatively) the largest foreflippers throughout life (Renous, Rimblot-Baly, Fretey & Pieau, 1988), and being highly streamlined. Davenport (1987) reported that hatchling leatherbacks could not swim backwards, while Renous & Bels (1993) demonstrated that juvenile leatherbacks (250-6000 g) use synchronized foreflipper action even when swimming slowly, only deviating from full synchrony when turning; they confirm that juveniles, like hatchlings, do not swim backwards or exhibit dog-paddle locomotion.

Green turtles appear to be faster-swimming and to use the foreflippers more than either loggerheads or ridleys; Zangerl (1980) rates cheloniid pelagic specialization in the order hawksbill > green > loggerhead > ridleys. Table 1 summarizes the known swimming modes of the living sea turtles so far studied (data for hawksbills and Kemp's ridleys are limited; no data for flatbacks appear to be available). There appear to be strong inverse correlations between anatomical specialization and flexibility of locomotion; it is also apparent that the 'Carettini' form a natural grouping of swimming styles as well as of general anatomy. Zangerl (1980) regarded ridleys as showing less pelagic specialization (from an anatomical viewpoint) than all other living sea turtles; the results presented here reinforce this conclusion.

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OBITUARY

PROFESSOR GEOFFREY A. D. HASLEWOOD
1910-1993

Professor Geoff Haslewood, Honorary Member and great friend of the British Herpetological Society, died in Worthing hospital on October 15th 1993. He will be greatly missed by his family and many friends both within and outside herpetology.

Geoff Haslewood had a long and distinguished career in the biochemical sciences before becoming actively involved with the BHS. His central interest was bile salts, a field in which he became the acknowledged world expert and which led to many honours. At the age of only 39 he was appointed to the first Chair of biochemistry at Guy's Hospital Medical School, a post he held until his retirement in 1977. Geoff was an active Member of the Biochemical Society, and its Chairman from 1969-1971; in the last year of his life he was made an Honorary Member of that learned body. Very unusually for a non-Japanese, Geoff was also an Honorary Member of the Japanese Biochemical Society. A major theme of his research concerned the relationship between the chemical structures of bile salts and evolution; indeed, Geoff Haslewood pioneered the study of molecular evolution which in these days of genetic engineering has become so fashionable (but much more expensive) with DNA and proteins.

After retiring from Guy's as an Emeritus Professor, Geoff and his wife Elizabeth turned their considerable energies towards the second love of their lives: herpetology and, in particular, lizards. My earliest encounter with them I remember very well, at the first (and last!) European Herpetological Symposium at Oxford in 1980. At that time little research had been done on natterjack toads, and following my delivery of a short and rather inconclusive talk on the subject I was promptly approached by Geoff along the lines of "Why don't you find out what is really going on, because the possibilities are obviously testable?" This Symposium marked the beginning of Geoff and Beth's active involvement within the BHS, and shortly afterwards both joined the Conservation Committee. Two years later Geoff became Chairman of this Committee, a role he fulfilled with great vigour and success from 1983-1987 inclusive. It was during this period that the Conservation Committee blossomed to include a financial turnover of tens of thousands of pounds and the employment, for the first time in the Society's history, of a full-time Conservation Officer. One highlight, of which Geoff was particularly proud, was a proper and systematic survey of smooth snakes in Britain carried out largely under his direction and subsequently published in the *Herpetological Journal*. He will be long remembered as an effective Chairman in every sense, from his succinct and disciplined control of meetings to his tactful but persistent negotiation with friends and foes of conservation alike.

Geoff remained an active member of the Conservation Committee long after his Chairmanship ended. He and Beth took over responsibility for the leaflets published by the Committee, and both continued to monitor sand lizard sites in summer as well as turning out on the clearance tasks organised every winter on the heathland homes of our endangered reptiles. Indeed, it was an inspiration to all of us to see Geoff working so hard on cold winter days well into his eighth decade. It took an incapacitating stroke in July of 1992 to force an unwelcome end to this otherwise irrepressible man's outdoor activities, but Geoff was making a slow and steady recovery from this blow when he was suddenly and unexpectedly taken ill again just a few days before he died.

It is not just for his achievements, impressive though they were, that Geoff will be remembered. Here was a kind, thoughtful and delightfully provocative man whose contribution was as much to *bonhomie* and the well-being of those around him as to academia and practical matters.The summer sessions of the Conservation Committee, so graciously hosted by Geoff and Beth (and often assisted by their daughters too) at their Sussex home and in the company of Geoff's beloved wall lizards, became renowned as the highlight of the Committee's year. We will all miss him dearly, and extend our deepest sympathies to Beth and to his daughters Sally and Barbara.

Trevor Beebee
University of Sussex

ANNOUNCEMENT

WORKSHOP ON POPULATION BIOLOGY OF AMPHIBIANS

A workshop on the Population Biology of Amphibians will be held in Vienna from 14-17 September 1994. The programme will include round-table discussions, posters presentations, a field trip, and a few invited lectures. Persons interested in participating please contact Dr Walter Hödl or Dr Günther Gollmann, both at the Institut fur Zoologie der Universitat Wien, Althanstr. 14, A-1090 Wien, Austria (Fax: +431 31336700).

THE HERPETOLOGICAL JOURNAL

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 œrst 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 l; 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 identiœed as (a), (b), etc. The orientation and name of the œrst 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 œrst 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.

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   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.

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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.

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Volume 4, Number 2 1994

CONTENTS

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