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

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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.

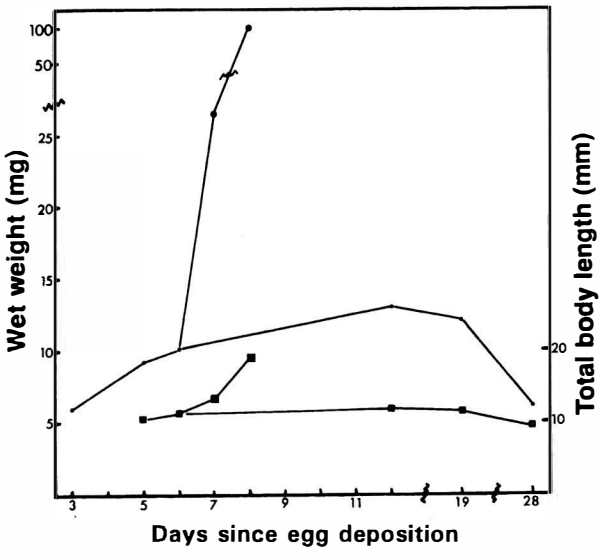

FIG. 1. Tadpole growth. Small symbols = tadpoles kept in foam; large symbols = tadpoles transferred to water with food. Circles = wet weights; squares = total body length; data from clutch 6, Table 1. Each point represents a mean value for several tadpoles.

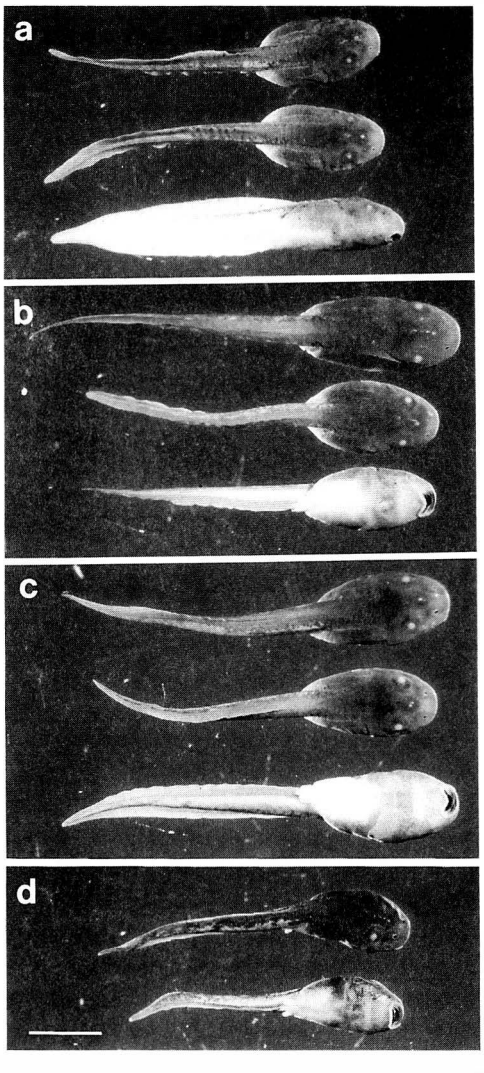

FIG. 2 Whole *L. fuscus* tadpoles from a single clutch of eggs, kept in foam for different times. (a) 5 days after deposition, (b) 12 days, (c) 19 days, and (d) 28 days. Scale line = 2mm

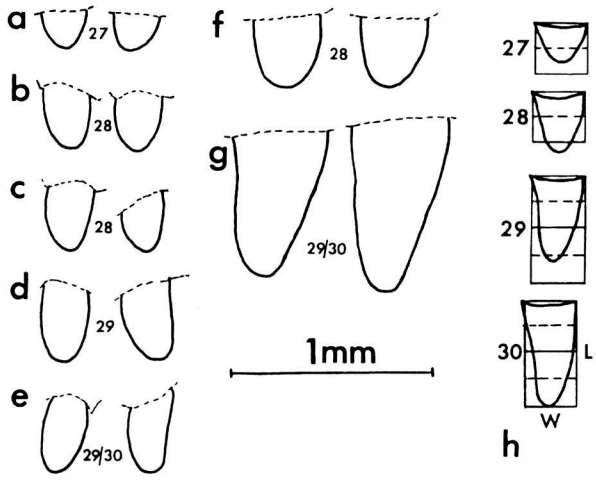

FIG. 3. Camera lucida drawings of hindlimb buds of *L. fuscus* tadpoles from a single clutch of eggs with Gosner stage numbers. (a)-(e) kept in foam for 5, 6, 12, 19, and 28 days post egg deposition, respectively. (f)-(g) tadpoles transferred to water with food at 6 days and fixed after 1, 2 days, respectively. (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.

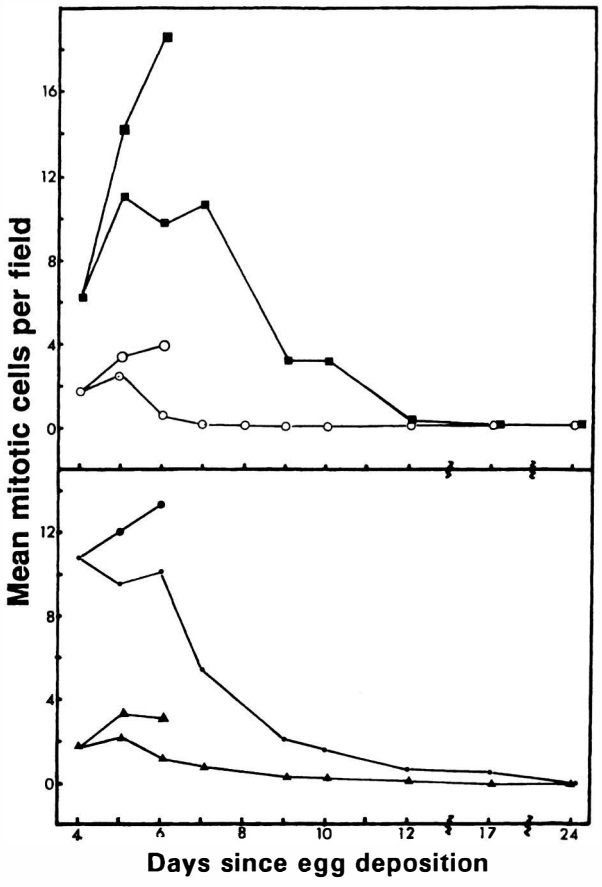

FIG. 4. Mitotic counts from different *L. fuscus* tadpole tissues. Small symbols = tadpoles kept in foam; large symbols = tadpoles transferred to water with food. Each point represents a mean value from several tadpoles. Filled circles = brain; triangles = limb epidermis (cells in field - 90); squares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

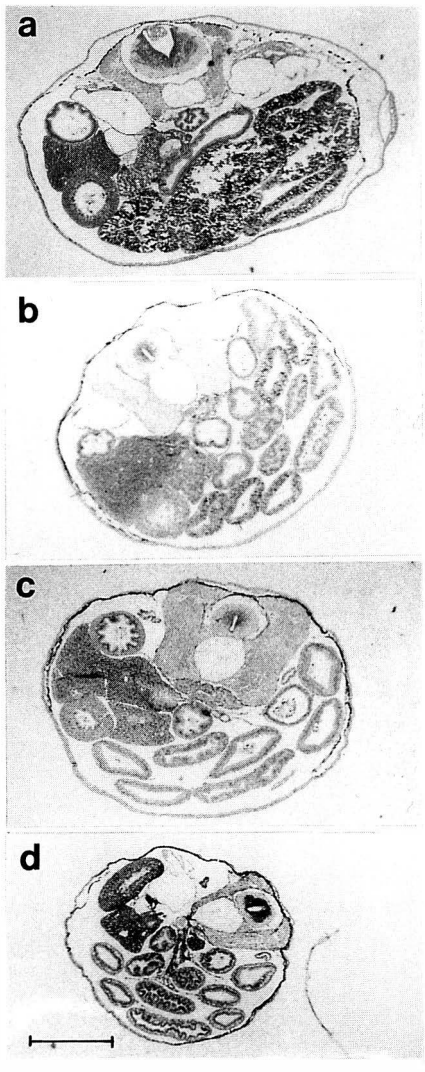

FIG. 5. Low power micrographs of haemalum and eosin-stained sections taken through the abdominal area of *L. fuscus* tadpoles from the same clutch of eggs, kept in foam for different times. (a) 5 days after egg deposition; (b) 12 days; (c) 19 days; and (d) 28 days. This region of the abdomen was used 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.

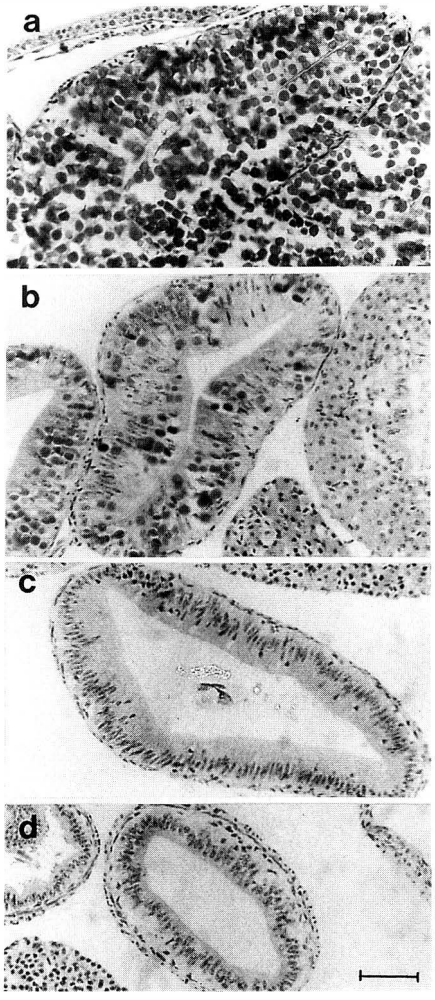

FIG. 6. High power micrographs of the gut sections labelled in Fig. 1. (a) 5 days after egg deposition - cells tall and packed with yolk granules; lumen occluded. (b) 12 days - cells lower, yolk still fairly abundant; lumen more open. (c) 19 days - cells lower still,one or two yolk granules only; lumen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

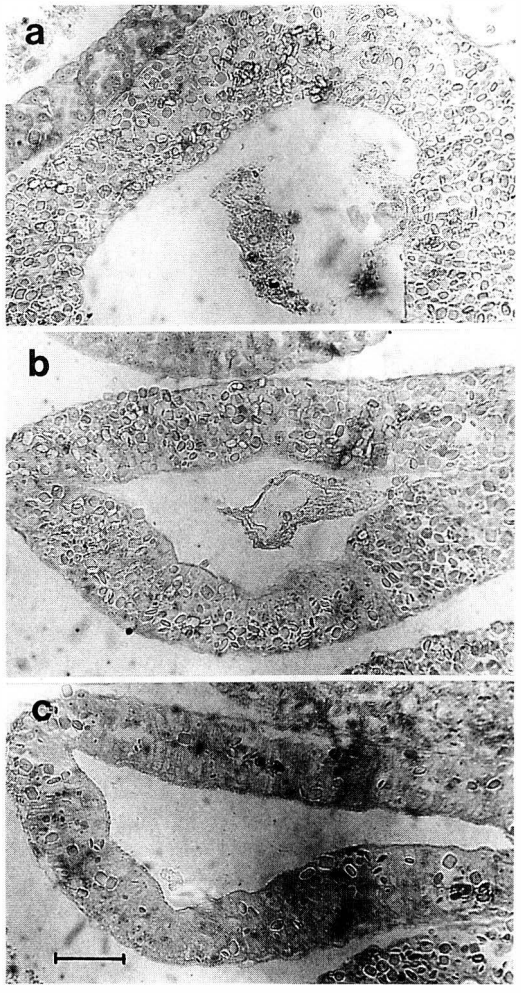

FIG. 7. High power micrographs of gut sections, equivalent to those labelled in Fig. 1, but from *L. fuscus* tadpoles transferred to water with food at stage 25 for (a) 1 day, (b) 2 days, and (c) 3 days. Scale line = 50 μm.

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

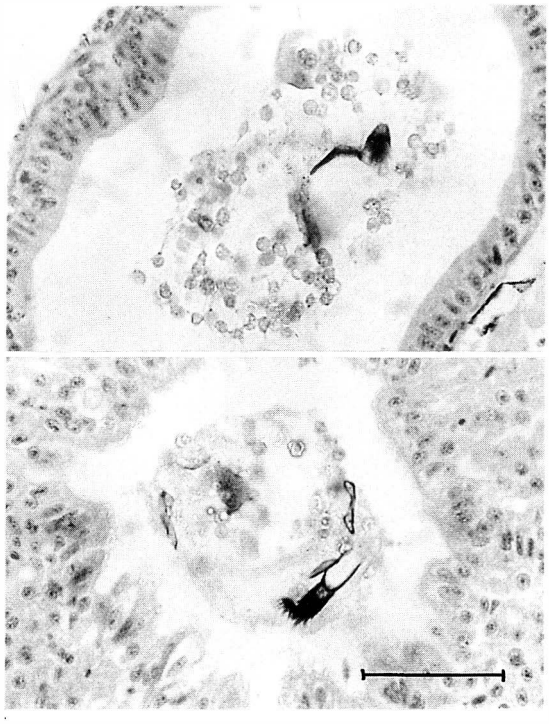

FIG. 8. High power micrographs of *L. fuscus* tadpole intestine sections to show tadpole teeth (dark, jagged structures) and single-celled micro-organisms (small round structures) amongst contents. This tadpole had been kept in foam two weeks after hatching. Scale line = 50 μm.

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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