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DEVELOPMENTAL ARREST IN *LEPTODACTYLUS FUSCUS* TADPOLES (ANURA: LEPTODACTYLIDAE) III: EFFECT OF LENGTH OF ARREST PERIOD ON GROWTH POTENTIAL

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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 hatching, the tadpoles make a new form of foam and, if no rain falls, enter a kind of developmental arrest. This may last around 30 days after egg deposition. In the experiments reported here, the ability of tadpoles to grow was tested after different periods of developmental arrest in foam nests. In the short term, tadpoles in foam for 15 days grew faster than those in foam 5 or 25 days (these grew at about the same rate). However, when raised to metamorphosis, a different pattern emerged. The longer tadpoles remained in foam, the slower they grew and the smaller the proportion that eventually metamorphosed. There was considerable variation between nests, with some showing high metamorphic potential 30 days after deposition but others low after only 18 days. Unexpectedly, size at metamorphosis varied with time spent in the nest. The longer tadpoles remained in the nest, the larger their mean size at metamorphosis, but also the greater their variability in size at metamorphosis. Some of the large tadpoles differed in shape from normal. Tadpoles allowed to grow soon after nest deposition grew rapidly to metamorphose at relatively smaller size and low variability. The significance of these results for the success of the developmental arrest strategy is discussed.

INTRODUCTION

Frogs of the *Leptodactylus 'fuscus'* species group (Heyer, 1978) deposit eggs in foam nests in burrows on land, near sites of temporary pools, generally in advance of heavy rains. In Trinidad, where we have studied *L. fuscus*, the wet season lasts about eight months, but is punctuated by dry spells of varying length when temporary pools may dry out. In species investigated so far, eggs develop past hatching but, in the absence of heavy rain, the tadpoles enter a form of developmental arrest (see Downie, 1994*a,b* for a detailed account of this phenomenon in *Leptodactylus fuscus* and for a review of our knowledge of other '*fuscus*' group species). If heavy rains fall during this period, the tadpoles are washed from their burrows and begin feeding. This mode of reproduction appears to have some advantages: when heavy rains fall, *L. fuscus* tadpoles enter the pool and are able to feed immediately, whereas most other amphibian species using the same pools are just beginning to deposit eggs. This allows *L. fuscus* tadpoles to feed on the eggs of other amphibians (Downie, 1988, 1990), and it also shortens the time that free-standing water must be available to allow metamorphosis to be reached, an important feature in areas with patchy and unpredictable rainfall.

This reproductive strategy may have some disadvantages. During the arrest period, the tadpoles remain active: they make a new kind of foam that replaces foam deposited by adults and actively wriggle within the foam (Downie, 1984, 1989). This activity requires resources: when the tadpoles enter developmental arrest, their gut endoderm is full of yolk granules; but after a few days, yolk content is greatly reduced, and is generally undetectable after two weeks. Furthermore, after three weeks of arrest, tadpoles begin to lose weight and eventually die, 27.5 days after egg deposition on average (Downie 1994*a*). It is clear that the ability to survive in the foam nest is limited and that if the adults breed too far in advance of significant rainfall, the entire clutch can be lost. Even before tadpoles die, they may have declined in body condition to a point from which they cannot recover. The experiments described in this paper were designed to test this prediction. Tadpoles were kept in foam for different periods of time, then their growth potential was assessed in two ways: (1) by determining survival to metamorphosis, size at metamorphosis, and development time; and (2) by measuring growth rate when given access to water and food.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF FOAM NESTS

Foam nests of *L. fuscus* were collected in July and August 1991 and 1993 from several sites in Trinidad, West Indies, mainly from a drainage ditch beside the main road to Piarco Airport in 1991, and from a ditch beside the recreation ground at Lopinot Village in 1993. At both sites the grass growing in and around the ditches was kept short, allowing easy access. The ditches filled quickly with water after heavy rain, but soon dried out if not replenished. Searches for foam nests were performed by inserting a spoon handle into

the mud of the side of the ditch, near the base, when the ditch was dry. Nest holes are generally not visible because they are plugged by the parents after deposition, but at Lopinot, a few incompletely plugged nests were found.

Only recently constructed nests with tadpoles at pre-foam-making stages were used, i.e. prior to Gosner (1960) stage 25. This is because it was important to know the time of egg deposition. Once tadpoles have entered developmental arrest, they do not progress beyond stage 28-29, and it is therefore impossible to tell the time of egg deposition from normal staging criteria (though it can be assessed approximately by histology: see Downie, 1994*a*)

After collection, nests were maintained in the laboratory on moist tissue paper in 250 ml polythene tubs, with the lids loosely attached to allow respiration but prevent evaporation. The tissue paper was replaced every few days. In Trinidad, the laboratory air temperature ranged from 27- 29°C. We have not attempted to monitor burrow temperature fluctuations in the field, but burrows are usually in the shade, with most eggs several centimetres below the mud surface, where they will be cooler than soil surface temperature. Because of the long-term nature of some of the experiments, some nests were brought to the laboratory in Glasgow, where they were maintained in an aquarium at an air temperature of 24-26°C. No attempt was made to regulate the lighting regime in either laboratory, since earlier experiments had shown that tadpoles make foam equally well in the dark and in the light (Downie, unpublished observations). There was no evidence that transportation of foam-making tadpoles to Glasgow caused any ill-effects.

Experiment 1: Growth to Metamorphosis

For this experiment, 20 foam nests were used, 8 in Trinidad and 12 in Glasgow. At intervals after collection (see Table 1 for times), 13 tadpoles were removed from each foam nest. Three of these were fixed in Bouin's fluid and later wet-weighed to 0.1 mg using a Sartorius Research balance: this established the state of the tadpoles at the start of the experiment. The remaining 10 tadpoles were transferred to 2 litre open polythene tubs containing 1500 ml dechlorinated copper-free tap water. Each tub was constantly aerated, and tadpoles were fed daily with crumbled fish flakes. The water was changed every 5-6 days. In Trinidad, water temperature was fairly constant at around 26°C, whereas in Glasgow it remained at 22-23°C.

Tadpoles were allowed to grow until they began to metamorphose, defined as the day of forelimb emergence (Gosner stage 42). On this day, metamorphosing tadpoles were wet-weighed to 0.01g. In the 1993 Glasgow series, after recording the wet weight at metamorphosis, tadpoles were fixed in Bouin's fluid. Snout-vent lengths were later measured to 0.1 mm using a binocular microscope and callipers. After this, tadpoles were dried in an oven at 80°C and dry weights were recorded to 0.1 mg. Lengths and dry weights were not measured in the 1991 experiment.

Time of death and approximate size were recorded for tadpoles that died during the experiment. These observations were not completely accurate because dead tadpoles were often consumed by the survivors.

The following data were recorded: proportion of tadpoles that reached metamorphosis; time taken to reach metamorphosis; and dry weight, wet weight, and length at metamorphosis.

Experiment 2: Short-term Growth Response

For this experiment, carried out in Trinidad (1993), six foam nests were used. At regular intervals after foam nest collection, samples of tadpoles were withdrawn as follows to assess their early response to growth conditions. For each foam nest, two 2 litre polythene tubs containing 1500 ml dechlorinated and aerated tap water were set up. To one tub, eight tadpoles were added and fed daily on crumbled fish flakes. Four tadpoles were removed and fixed in Bouin's fluid after one day, and the remainder after two days. To the other tub, 12 tadpoles were added but not fed. Four tadpoles were removed and fixed after 1, 2 and 6 days. In addition, on the day of setting up the tubs and six days later, four tadpoles were removed from the foam nests and fixed as controls. Laboratory air temperature was 28-29°C. Preserved tadpoles were later measured to 0.1 mm using an eyepiece graticule in a Wild M5 binocular microscope at X60 overall magnification; wet and dry weights were measured to 0.1 mg.

Histological Processing and Examination

Abnormal tadpoles were fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned at 7 μm and stained with haemalum, eosin and alcian blue; they were examined with a Wild M20 microscope.

RESULTS

Growth to Metamorphosis

The aim of this experiment was to compare the ability to reach metamorphosis of tadpoles kept for different times in their foam nests. Time since egg deposition was known to 1-2 days for all foam nests used. The variable times since deposition used in the comparison differed for the Trinidad and Glasgow experiments because of collection and transportation requirements. In Trinidad, six of the foam nests used had tadpoles left after all growth experiments were set up: mean survival time post-deposition for these tadpoles was 17.5 days; in Glasgow, mean survival for seven nests of tadpoles was 32.4 days. In the Trinidad experiment, the longest time since deposition before tadpoles were set to grow was 17 days; in Glasgow, 32 days: the latter group was therefore close to the end of the survival capacity of these tadpoles in foam.

TABLE 1. Percentage of tadpoles reaching metamorphosis. Time class is the time between egg deposition and tadpoles entering water. *t*-test result given for Trinidad comparison; ANOVA performed for Glasgow comparison on arcsin-transformed percentages. ** $P < 0.01$. *Post-hoc* comparisons were C with D, E and F; D with E and F; E with F: superscripts which differ indicate significant differences between treatments ($P < 0.05$).

	Series	Time class (days) mean±SD	No. of group	% reaching metamorphosis Mean±SD	% reaching metamorphosis Range	Statistics
A	Trinidad	5.6±0.9	8	95.0±7.6	80 - 100	$t = 1.43$ NS
B		12.8±3.4	4	87.5±12.6	70 - 100	
C	Glasgow	9.9±3.0	9	96.7±5.0[a]	90 - 100	$F_{3,34} = 5.5$**
D		17.5±1.5	12	85.0±23.5[a]	20 - 100	
E		26.0±1.5	11	60.7±25.5[b]	0 - 90	
F		31.0±0.6	6	41.7±20.4[b]	10 - 70	

TABLE 2. Relationship between metamorphic potential and the condition of tadpoles in five foam nests. Metamorphic potential is given as the percentage of tadpoles reaching metamorphosis from an initial group of 10 (Column A). Tadpole condition is given as the mean wet weight (mg±SD) of a sample of 3-4 tadpoles taken from the nest at the same time as 10 tadpoles started growth to metamorphosis (Column B).

	Nest number									
	1		2		3		4		5	
Mean days since egg deposition	A	B	A	B	A	B	A	B	A	B
9.9	100	12.2±0.9	100	15.7±0.7	100	10.9±0.5	100	10.3±0.2	90	11.5 ±0.5
17.5	20	8.1±1.5	100	16.6±1.1	90	11.3±1.1	90	11.6±0.8	80	10.9±1.3
26.0	0	6.6±0.7	90	11.7±2.0	80	7.6±2.3	50	6.4±1.4	60	7.5±1.4
31.0	10	6.4±1.0	70	7.8±0.7	40	6.1±1.2	50	5.0±0.6	50	5.2±1.1

Proportion of tadpoles reaching metamorphosis. In both the Glasgow and the Trinidad series, there was a trend towards declining ability to reach metamorphosis the longer tadpoles remained in foam (Table 1). For tadpoles set up just after foam-making began, only seven out of 170 failed to reach metamorphosis; but tadpoles entering water about 30 days after egg deposition had less than a 50% chance of reaching metamorphosis. Observations on the time and stage of death showed no particular trend. Some died very small; others died near metamorphosis, and others in between. In most cases, there was no obvious cause of death. In a few, tadpole development was clearly abnormal: one had a twisted vertebral column and could not swim properly; in several, the body cavity became considerably distended, apparently full of air, and the tadpoles tended to swim sluggishly at the surface, before eventually dying. Histological examination of sections of such tadpoles showed a highly abnormal intestine, set to one side rather than filling the whole abdominal cavity and with a contracted lumen. Individual cells, however, looked healthy and the cause of this abnormality was not apparent.

The trend shown by the complete data set also occurred in individual foam nests. Results from 5 Glasgow nests are shown in Table 2. There was considerable variability between foam nests, with nest 1 showing a particularly steep decline in metamorphic potential, but nest 2 having high metamorphic potential even 31 days after deposition.

Table 2 also shows the mean wet weights of tadpoles taken from the foam nests at the times tadpoles were transferred to water to allow them to grow. For each nest, wet weight declined the longer tadpoles remained in foam, but the rate of decline differed from nest to nest: it was steepest for nest 1, correlating with the steepness of decline of metamorphic potential in this nest and shallowest for nest 2, which had the longest duration of high metamorphic potential. The remaining three nests were intermediate between these extremes in both weight and metamorphic potential decline.

Time taken to reach metamorphosis. The data on time taken to reach metamorphosis are shown (Table 3) as (1) time taken for the first tadpoles to reach metamorphosis, (2) time between the first and last metamorphosis - a measure of whether all tadpoles in a

TABLE 3. Time (days) taken by tadpoles to reach metamorphosis after being put in water with food: (a) time taken for the first tadpole in each group to reach metamorphosis; (b) time between first and last metamorphosis in a group; (c) time for all tadpoles in a group to reach metamorphosis. Superscript 1 denotes data sets where the percentage of tadpoles reaching metamorphosis was sometimes less than 80%. Figures in brackets show the data for those cases where 80% or above did metamorphose. t-test results given for Trinidad comparisons. ANOVA results given for Glasgow comparisons. $*P < 0.05$; $**P < 0.01$. *Post-hoc* comparisons were C with D, E and F; D with E and F; E with F. Superscripts which differ indicate significant differences between treatments ($P < 0.05$).

		Series	Mean days since egg deposition	No. of groups	No. days (mean±SD)	Statistics
(a)	A	Trinidad	5.6	9	16.8±3.2	$t = 0.51$ NS
	B		12.8	7	16.0±2.6	
	C	Glasgow	9.9	9	$18.6±2.9^a$	
	D		17.5	13	$29.5±9.7^b$	$F_{3,34} = 4.39*$
	E		26.0	10	$29.5±9.2^b$	
	F		31.0	6	$32.8±12.1^b$	
(b)	A	Trinidad	5.6	8	8.0±3.8	$t = 0.79$ NS
	B		12.8	3	6.0±2.0	
	C	Glasgow	9.9	9	$14.2±8.6^a$	
	D		17.5^1	12	$42.0±19.9^b$	
				(10)	(47.2±17.4)	$F_{3,32} = 8.05$
	E		26.0^1	10	$41.8±15.0^b$	
				(4)	(45.3±18.0)	
	F		31.0^1	5	$57.6±22.2^b$	
				(0)		
(c)	A	Trinidad	5.6	8	19.6±4.1	$t = 1.27$ NS
	B		12.8^1	4	16.7±2.4	
				(3)	(17.2±2.9)	
	C	Glasgow	9.9	9	$24.3±6.9^a$	
	D		17.5^1	12	$46.5±12.1^b$	
				(10)	(47.6±11.8)	$F_{3,32} = 3.45*$
	E		26.0^1	10	$48.2±12.2^b$	
				(4)	(42.0±10.0)	
	F		31.0^1	5	$61.1±22.2^b$	
				(0)		

group developed together, or whether they were very spread out, and (3) mean time for all to reach metamorphosis: since a high proportion of tadpoles in the higher time classes failed to reach metamorphosis, these data are presented in two ways - mean times for all that metamorphosed, and mean times for groups where 80% or more did metamorphose.

The minimum time taken for any tadpole to reach metamorphosis was 12 days (17 days after egg deposition) and the maximum recorded was 143 days (including 32 days in foam, this was 175 days after egg deposition). Inspection of Table 3 shows that the longer tadpoles remained in foam, the longer it took to reach metamorphosis, whether the measure was of the first tadpole to reach metamorphosis, or the mean time for all tadpoles. The discrepancy concerns the two Trinidad groups where times to metamorphosis are not distinguishable. Compared to the Glasgow groups, all the Trinidad tadpoles had been kept in foam for a relatively short time.

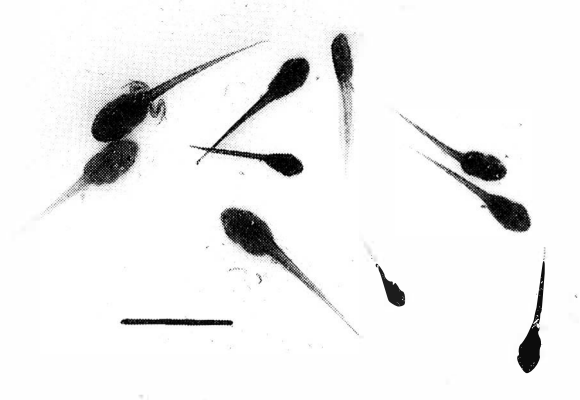

FIG. 1. Group of 10 tadpoles from a single clutch grown for 36 days, after 20 days kept in a foam nest. The largest in the group is near metamorphosis; the smallest has hardly grown at all. Scale bar = 20 mm.

TABLE 4. Mean wet weights, in grams, for all tadpoles in each group, on reaching metamorphosis. Superscript 1 denotes data sets where the proportion of tadpoles reaching metamorphosis was sometimes less than 80%. Figures in brackets show the results for those cases where 80% or above did metamorphose. t - test results given for Trinidad comparisons. ANOVA results given for Glasgow comparisons. $*P < 0.05$. *Post-hoc* comparisons were C with D, E and F; D with E and F; E with F. Superscripts which differ indicate significant differences between treatments ($P < 0.05$), except mean wet weights D and E NS.

	Series	Mean days since egg deposition	No. of groups	Mean wet wt.±SD	Statistics	Mean std. dev. ±SD	Statistics
A	Trinidad	5.6[1]	9	0.24±0.04	$t = 0.014$ NS	0.04±0.01	$t = 0.49$ NS
			(8)	(0.25±0.04)		(0.04±0.01)	
B		12.8[1]	5	0.24±0.04		0.04±0.02	
			(3)	(0.24±0.04)		(0.03±0.01)	
C	Glasgow	9.9	9	0.25±0.02[a]	$F_{3,32} = 4.75*$	0.03±0.0[1a]	$F_{3,32} = 4.74*$
D		17.5[1]	12	0.29±0.03[a]		0.08±0.03[b]	
			(10)	(0.29±0.03)		(0.08±0.03)	
E		26.0[1]	10	0.32±0.08[b]		0.08±0.03[b]	
			(4)	(0.28±0.02)		(0.08±0.02)	
F		31.0[1]	5	0.37± 0.10[b]		0.12±0.10[b]	
			(0)				

TABLE 5. Mean dry weight (mg) for all tadpoles in each group, on reaching metamorphosis (measured only in Glasgow, 1993 experiment). Superscript 1 denotes data sets where the proportion of tadpoles reaching metamorphosis was sometimes less than 80%. Figures in brackets show the results for those cases where 80% or above did metamorphose. Means compared by ANOVA. $**P < 0.01$. *Post-hoc* comparisons were A with B, C and D; B with C and D; C with D. Superscripts which differ indicate significant differences between treatments ($P < 0.05$).

	Mean days since egg deposition	No. of groups	Mean dry wt. ±SD	Statistics	Mean std. dev. ±SD	Statistics
A	9.9	7	37.5±3.9[a]	$F_{3,24} = 6.83**$	5.2 ±1.4[a]	$F_{3,24} = 14.94**$
B	17.5[1]	10	48.7±6.0[b]		16.0± 5.4[b]	
		(9)	(47.7±5.4)		(17.0±4.7)	
C	26.0[1]	6	53.2±8.6[b]		13.9±2.9[b]	
		(3)	(47.1±5.3)		(14.8±3.7)	
D	31.0[1]	5	(54.4±11.9[a])		12.5±4.9	

As expected from the laboratory temperature differences, all times in Glasgow were somewhat longer than in Trinidad. In addition, the longer tadpoles remained in the nest, the more variable was their time to reach metamorphosis, measured both by the time between first and last metamorphosis and by the standard deviations of the mean times. One group set up soon after egg deposition all metamorphosed over two days, whereas it took 95 days from the first to last in one group set up after 31 days in the nest.

This variability is evident in Fig. 1, a photograph of a complete group of tadpoles set up after 26 days in the nest, just as the first tadpole was beginning to metamorphose after 36 days in water with food. The smallest tadpole here was hardly bigger than when it was removed from the foam nest. Only three of this group eventually reached metamorphosis.

Sizes of tadpoles on reaching metamorphosis. The sizes of tadpoles at metamorphosis were measured in three ways: snout-vent length (not shown), wet weight (Table 4), and dry weight (Table 5). As with the time-to-metamorphosis results, there was no significant difference between the two Trinidad groups. However, in the Glasgow experiments, all measures of size at metamorphosis showed that size was greater the longer the time tadpoles had spent in the nest. In addition, variation in size at metamorphosis also increased with time in the nest. For example, from one foam nest, dry weights at metamorphosis changed from 35-48 mg (mean=40, n=9) for the group earliest out of the nest to 45-96 (mean=61, n=6) for the group last out of the nest. That size at metamorphosis should increase the longer tadpoles remained in the foam nest was a totally unexpected result.

TABLE 6. Rate of growth to metamorphosis measured as dry weight (mg) at metamorphosis, divided by the number of days taken to reach metamorphosis after access to food. Superscript 1 denotes data sets where the proportion of tadpoles reaching metamorphosis was sometimes less than 80%. Figures in brackets show the results for those cases where 80% or above did metamorphose. Means compared by ANOVA. NS $P > 0.05$.

Mean days since egg deposition	No. of groups	Mean dry wt./day ±SD	Statistics
9.9	7	1.60±0.32	$F_{3,24}$= 2.49 NS
17.5[1]	10 (9)	1.16±0.37 (1.07±0.25)	
26.0[1]	6	1.27±0.43 (1.27±0.62)	
31.0[1]	5 (0)	1.01±0.53	

Rate of growth of tadpoles in reaching metamorphosis. Table 6 gives the rate of growth to metamorphosis in terms of dry weight gain per day for the different groups, using only data from the Glasgow 1993 experiment. The results show that the longer tadpoles remained in foam, the slower they grew once they were given access to water and food.

SHORT-TERM GROWTH RESPONSE

The results of the short-term growth experiments (Table 7) are based on pooled data from six different nests. Tadpoles in foam nests declined gradually and continuously in dry weight over the 31 day period monitored after egg deposition, ending at 38% of starting weight.

When added to water with food, tadpoles grew rapidly, but the rate of growth varied according to the length of time spent in the foam nests. Intriguingly, the fastest growth in dry weight was not in the earliest group (= 5 days after egg deposition) but in the second group (= 15 days); and in the third group (= 25 days) growth rate, both in percentage terms and absolute terms (amount of dry mass added per day), was similar to the earliest group. After two days growth, tadpoles in the third group were smaller than the others, but they started from a lower initial weight. Wet weight and length measurements (not shown) showed essentially the same pattern of changes. It should be noted that not all the growth recorded is tissue growth: for these tadpoles, unassimilated food in the gut is a significant proportion of body weight.

When added to water with no food, to test the effect of tissue hydration alone, there was no significant change in dry weight in any group of tadpoles. However, in the earlier groups, body length and wet weight did increase continuously over the six days in water. In the third group there was an initial increase in wet weight and body length over the first day but no increase over the following five days.

DISCUSSION

Body condition of *Leptodactylus fuscus* tadpoles kept in their foam nests for progressively longer periods was investigated. Previous work (Downie, 1994*a*) established that tadpole weight declined the longer tadpoles remained in the nest and that they eventually died if they did not get access to food and water. Mean survival time post-deposition was 27.5 days (range 19-33; 6 nests). In the present study, mean survival time was 32.4±2.7 days (range 27-36; 7 nests; maintained in

TABLE 7. Short term growth response: dry weight measurements (mg; mean ± SD; number sampled in brackets). ANOVA results given for each group.*** $P < 0.001$. *Post-hoc* comparisons were A with B, C and D. B with C and D. Superscripts which differ indicate significant differences between treatments ($P< 0.05$).

Group	Days since egg deposition (mean±SD)	Tadpoles taken from foam nest (controls)		Tadpoles in water with no food			Tadpoles in water with food		Statistics
		Day 0 A	Day 6	Day 1 B	Day 2	Day 6 C	Day 1 D	Day 2	
1	5.3±0.8	2.1±0.5[a] (25)	1.9±0.4 (18)	2.3±0.4[a] (24)	2.4±0.7 (23)	2.2 ± 0.6[a] (25)	3.2±0.5[b] (24)	5.6±0.8 (24)	$F_{6,157}$= 21.2***
2	15.3±0.8	1.5± 0.4[a] (22)	1.4±0.4 (17)	1.6± 0.3[a] (18)	1.6± 0.4 (19)	1.7±0.7[a] (15)	3.9 ± 0.6[b] (22)	6.9±1.8 (22)	$F_{6,129}$=123.2***
3	25.3±0.8	1.0±0.3[a] (21)	0.8±0.3 (23)	1.3±0.3[a] (21)	1.2 ± 0.3 (19)	1.0± 0.4[a] (17)	2.0±0.5[b] (22)	3.9±1.0 (22)	$F_{6,139}$=92.9***

Glasgow). The longer survival time may partly be explained by lower maintenance temperature, and partly by improved husbandry of the foam nests. The results reported here extend the earlier work to include a study of the responses of tadpoles given access to water and food after different times in the nest.

The longer tadpoles remained in their foam-nests, the less likely they were to reach metamorphosis, the longer it would take on average, and the slower they would grow. Within these broad trends were three more surprising findings. First, decline in metamorphic potential varied considerably from clutch to clutch. Second, within any one clutch, the longer tadpoles remained in foam, the more variable their metamorphic potential became. Third, the longer tadpoles remained in foam, the larger their mean size at metamorphosis became.

The second response measured was initial growth rate, for the period 1-6 days after tadpoles were given access to water and food. In water alone, no increases in dry weight occurred, but length and wet weight measurements revealed a difference in response according to the time spent in the foam nest. Tadpoles kept in foam up to 15 days after egg deposition retain some yolk reserves (Downie, 1994*a*) and in water alone, these tadpoles grew in body length and wet weight for the six days of the experiment, presumably converting the yolk into tissue, especially gut. Tadpoles kept in foam 25 days had no yolk left, and these tadpoles merely showed a one-day increase due to tissue hydration.

When tadpoles were given food, all growth measurements showed increases over two days, but rates differed according to time spent in foam. The fastest growth occurred 15 days after egg deposition with the rate being slower at five and 25 days. The most likely explanation of these results is that at five days, the food gathering and processing system is still relatively immature. Tadpoles are able to gather and utilize food, but not quite so effectively as somewhat older tadpoles. However, by 25 days, body weight has begun to decline, involving tissue breakdown to provide for metabolic needs: this may lead to a progressive decline in the effectiveness of food gathering and processing.

The most general result from this study is that the longer tadpoles remained in foam, the less likely they were to reach metamorphosis once they had access to food and water. In other words, they had declined in some manner in body condition. Part of the reason for this may be the decline in body weight experienced by tadpoles towards the end of the foam-making period. However, tadpoles at this stage given food and water were able to grow and in most clutches, some eventually reached metamorphosis, so the growth decline did not in itself mean that tadpoles were doomed to die. A possible additional factor contributing to the decline in metamorphic potential is infection: during the time in foam, various kinds of micro-organisms may become associated with the tadpoles and cumulatively lead to deterioration in physiological systems. Downie (1994*a*) noticed that the guts of tadpoles in foam contained large numbers of unicellular organisms; and tadpoles that died at various times after entering water often showed signs of fungal attack. There has been considerable controversy over the identity of the unicellular organisms commonly found in tadpole guts and over their role in the phenomenon of competitive growth inhibition (Beebee, 1995; Petranka, 1995) but their effects on non-growing tadpoles in foam nests are unknown.

There is now a considerable literature on the plasticity of tadpole development, based on the suggestion by Wilbur & Collins (1973) that many tadpole species may respond adaptively to their recent growth history - metamorphosing if this is slow, remaining to grow further in water if fast. Results from different studies have been variable, one showing the prime determining factor as early food supply (Leips & Travis, 1994), another that growth rate in the middle period is the main determinant (Hensley, 1993). In some cases, plasticity appears not to be adaptive (Tejedo & Reques, 1994); in others, as in a series of studies on the desert amphibian *Scaphiopus couchii* (Newman 1988, 1989, 1994), a clearly adaptive response to food, water and space has been demonstrated. These studies have all been on tadpole species that begin feeding immediately. The situation described here for *Leptodactylus fuscus*, with tadpoles remaining up to several weeks in the nest before starting to feed, is highly unusual. However, this factor - time in the nest before feeding - had an effect on future development under constant conditions: the longer the time in the nest, the longer the growth period, the larger the mean size at metamorphosis, and the more variable the time to, and size at, metamorphosis. Another variable was the speed of change in body condition which differed greatly from clutch to clutch.

Are any of these effects adaptive? We doubt that this question can be answered fully at present. However, knowledge is accumulating of unexpected adaptive responses to unpredictable environments and it is not unreasonable to suggest that for *L. fuscus* tadpoles that enter water early, the adaptive response is to regard conditions as generally good, allowing uniform growth to optimal size; but for those that remain longer and longer in foam, conditions may be perceived as patchy, encouraging something like a 'coin-flipping' response (Kaplan & Cooper, 1984) where some individuals choose rapid development to early, small size metamorphosis and others choose late, large size.

Another possibility worth investigating in this system is polyphenism. Pfennig (1992) found that *Scaphiopus multiplicatus* tadpoles develop as large carnivorous morphs or smaller omnivorous morphs, depending on resource availability and quality. The body shape of the large *L. fuscus* tadpoles reported here is similar to Pfennig's carnivores, while the smaller *L. fuscus* are like the omnivores. Again, the extremes of size in *L. fuscus* developed in response to longer peri-

ods of developmental arrest in foam, and could therefore involve some kind of developmental switch. Testing whether the proportion of large to small morphs is responsive to conditions and whether the two morphs differ in features other than size (as do the *S. multiplicatus* morphs) will be an objective of future research.

Although some of the life history features revealed by this study may be adaptive, the main result is the decline in metamorphic potential the longer tadpoles remained in the foam nest. It is possible that the results are partly artefactual: foam nests were maintained on damp tissue, rather than on mud; tadpoles were kept in aquaria and fed artificially. It will therefore be worth attempting to replicate this work under more natural conditions. If the results do reflect reality in the field, decline in tadpole condition and metamorphic potential must be a limitation on the success of the *L. fuscus* strategy of reproducing in advance of major rainfall. The magnitude on this limitation will vary with rainfall patterns.

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