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

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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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