OBSERVATIONS ON FOAM-MAKING BY *LEPTODACTYLUS FUSCUS* TADPOLES

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(Accepted 17.3.88)

ABSTRACT

Downie (1984) showed that recently hatched tadpoles of the ground nesting frog *Leptodactylus fuscus* make a foam which replaces the original nest foam made by the mating adults, but did not describe how it is made. The present results show that 1) foam-making is a communal activity: single tadpoles do not do it; 2) foam bubbles are made mainly by spitting movements of the mouth, but also by wriggling of the tail; 3) foam is probably stabilised by mucus secreted by buccal glands; precocious secretion by these glands may be an adaptation to foam-making; 4) components of foam making behaviour are shown by other tadpole species out of water, but the complete behaviour is specific to recently hatched *L. fuscus*.

INTRODUCTION

Frogs of the family Leptodactylidae deposit their eggs in foam nests, either in burrows on land, or floating on the surface of water. Downie (1984) reported that in the ground-nesting species *Leptodactylus fuscus* (Heyer, 1968) the hatched tadpoles, which may remain in the nest up to several weeks, depending on when the rains fall, themselves make a foam which replaces the original foam, if the tadpoles remain in the nest long after hatching. Downie noted that the ability to make foam wanes once tadpoles enter water, and also wanes the longer they remain in the nest, but did not report on how the tadpoles make foam, other than to surmise that it is made by whipping movements acting on secretions. The following paper investigates in detail how these tadpoles carry out this unusual activity.

MATERIAL AND METHODS

COLLECTION AND MAINTENANCE OF TADPOLES

Seven 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, in July 1984 and July 1987. In addition, because nests were hard to find in 1987 (due to tidying up of the site), two clutches of newly-emerged tadpoles were collected from freshly-flooded pools in the same area, removed from water, then placed on the surface of damp tissue in a polythene tub, to stimulate them to make foam.

It proved preferable to use pre-stage 27-28 tadpoles (staged by Gosner, 1960) from foam nests, since this allowed the tadpoles to be accurately aged. Once they reach stage 27-28, they remain at this morphological stage as long as they remain in the nest, but their ability to make foam progressively declines.

Foam-making tadpoles were maintained on the surface of moist tissue paper in small polythene tubs. For later stages, some tadpoles were transferred to tanks and fed on mixed grain powder and fish food.

For comparison, tadpoles of three other Trinidad species were collected: floating foam nests of *Physalaemus pustulosus* were collected from drainage ditches and puddles in St Augustine, and tadpoles hatching from these nests were grown in glass tanks; tadpoles of *Colostethus trinitatis* were collected from Tamana cave; tadpoles of *Bufo granulosus* were collected from puddles in St Augustine (see Kenny, 1969a, for species accounts and Harding, 1983, for nomenclature alterations).

BEHAVIOURAL OBSERVATIONS

To observe tadpole behaviour out of water, tadpoles were removed from foam or from tanks, washed, and placed singly or in groups on the surface of moist tissue in glass petri dishes, with the lids on. The behaviour of the tadpoles was then recorded at regular intervals, as described under Results.

STRUCTURAL ANALYSIS

After washing, tadpoles were fixed in Bouin's fluid (for light microscopy) or 2 per cent glutaraldehyde in phosphate buffer (for electron microscopy). Wax sections were stained with haematoxylin and eosin, or by the Periodic acid — Schiff's (PAS) method. Glutaraldehyde-fixed tissue was post-fixed with 1 per cent osmium tetroxide in phosphate buffer then processed by standard methods for examination with a Phillips 500 scanning electron microscope, or embedded in araldite resin for semithin and ultrathin sectioning. Semithin sections were stained with toluidine blue, and ultrathins with uranyl acetate and lead citrate before examination with an AEI 801 transmission electron microscope.

RESULTS

Behavioural Observations

Initiation of foam-making by L. fuscus *tadpoles*

The earliest hatchlings I saw were at Gosner stage 22, estimated as 3.5 days old (assuming egg deposition at the middle of the night). Since Gosner quotes stages 17-20 as hatching time for most species, it may be that *L. fuscus* hatches a little earlier than stage 22, but takes some time to wriggle free of foam. These early hatchlings were not able to make foam. This was established by washing tadpoles of stage 23-24, and, for comparison, tadpoles which had just reached stage 26-27, and placing them in a heap on moist tissue in polythene tubs. Stage 26-27 tadpoles made foam within 12 h; stage 23-24 tadpoles did not (results not shown).

Foam-making is a communal activity

My original observations (Downie, 1984) on foam-making involved many tadpoles placed together in a heap. An obvious question is: can single tadpoles make foam, or is this a group activity? To answer this, I placed washed stage 27-28 tadpoles on the surface of moist tissue in petri dishes in groups of 10, 5 and 3, or as individuals approximately 2cm from one another, then checked for the presence of foam after one and two days. The results are shown in Table 1. Groups as small as 3 did make foam, though less well than larger groups; individuals did not make foam at all.

Number in group	10	5	3	1
Number of trials	5	14	16	52
% showing foam after 1d	100	71	19	0
% showing foam after 2d	100	78	38	0

TABLE 1: Foam-making by groups or individuals of *L. fuscus* tadpoles on damp soil.

How foam is made

To find how tadpoles make foam, groups of 5 or 6 stage 27-28 tadpoles were removed from foam, washed, and placed in a heap on the surface of moist tissue in a petri dish. Using a Wild dissecting microscope, they were then observed at intervals until they had made a substantial amount of foam. The following features were noted:

1) Although a tadpole might occasionally move away from the group for a short time, by active wriggling, groups generally kept together.
2) Movements made by tadpoles included rapid wriggling of the whole body — sometimes several times, in quick succession — and jerking movements of the head or tail alone. Wriggles sometimes moved tadpoles up and over the other tadpoles in the group. Head movements were sometimes preceded by contractions within the pharynx, and usually ended with the mouth spitting out one or several bubbles, ranging from 0.07-0.8mm in diameter. Occasionally, a rapid tail wriggle produced a bubble in the surface moisture film.
3) Bubbles made soon after isolation of the tadpoles were generally unstable, bursting spontaneously or after tadpole wriggling movements. Bubbles made later were more stable. Sometimes, wriggling movements broke larger bubbles into smaller ones.
4) Tadpoles at the top of a heap were more likely to move than those at the bottom, and very commonly, several tadpoles moved together.

As an example, in one group of 5 tadpoles, observed at intervals over a 6 hour period, the first bubbles to be formed all burst quickly. By 2 hours, there were about 10 bubbles, but by 3 hours, 60 or so. In a 10 minute continuous observation period, made at 6 hours, there were 9 episodes of tadpole wriggling and spitting, most involving two or three tadpoles at a time. Only the bottom tadpole in the heap did not move during this time.

The specificity of foam-making behaviour

The observations reported in the previous sections suggest two further questions: 1) Why do isolated individual stage 27-28 tadpoles not make foam, while the same tadpoles in groups do; 2) is the behaviour shown by foam-making tadpoles specific to them?

To investigate the first question, I set up dishes of washed foam-making tadpoles on damp tissue, 5 or 6 to a dish, with the tadpoles either in a heap, or as isolated individuals, in order to look for differences in their behaviour. All tadpole movements were recorded for a number of 5 minute periods starting from one hour after setting up and carrying on for up to 10 hours. The different kinds of movements — head shakes, tail flicks, whole body wriggles — were all counted together simply as 'movements'. The results are shown in Table 2. The movements the tadpoles made are presented in two ways: 1) as the mean number of movement 'episodes' per tadpole in each 5 minute period, irrespective of the number of tadpoles that moved at any one time; a movement episode begins when one tadpole starts to move and continues till that and other tadpoles cease moving. 2) as the mean number of tadpoles that moved during each movement episode. A comparison of the figures for groups and individuals shows both that tadpoles in a group are more likely to move in a 5 minute period than individual tadpoles ($P<0.001$, Student's t test) and that in any episode of movement, several tadpoles in a group tend to synchronise their movements, whereas isolated tadpoles show no such tendency.

To investigate the second question, I compared the behaviour of foam-making *L. fuscus* tadpoles with that of later stage *L. fuscus*, and that of two other Trinidad tadpole species, *Physalaemus pustulosus* and *Colostethus trinitatis*, chosen because previous studies (unpublished) had shown these tadpoles to survive some time out of water.

Later stage *L. fuscus* and *P. pustulosus* tadpoles were quite active when first removed from water, with wriggling movements and occasional mouth spitting, forming a few short-lived bubbles, but they soon became rather inactive, whether in groups or singly. In both cases, groups tended to stay together. A small

Tadpole type, number and arrangement	Number of observation periods	Mean number of movement episodes/ tadpole/period	Mean number of tadpoles moving/ movement episode
Foam-making *L. fuscus* 5 or 6 in a heap	13	1.38	1.92
Foam-making *L. fuscus* 5 or 6 individuals	11	0.63	1.01
Later stage *L. fuscus* 5 in a heap	4(3*)	0.3	2.5
Later stage *L. fuscus* 5 individuals	3	0.4	1.17
Colostethus trinitatis 5 individuals	14	2.10	1.19
Physalaemus pustulosus stage 30, 5 in a heap	7(4*)	0.14	1.0
Physalaemus pustulosus stage 30, 5 individuals	7(2*)	0.4	1.0

TABLE 2: Analysis of movements made by several species of tadpoles out of water, during a series of 5 minute observation periods. * = observation periods with no movement.

number of 5 minute observations were made after the initial active phase, and these are recorded in Table 2. Overall activity was very low compared to foam-making *L. fuscus*.

C. trinitatis tadpoles behaved very differently. They remained active throughout a 10 hour experiment. Tadpoles did not stay together if set up in groups. Movements were often very active wriggles, even jumps that could carry a tadpole right across the dish. Mouth spitting was not seen. Five minute observation results are shown in Table 2, and demonstrate that these were the most active tadpoles observed. Synchronisation of movement was low, perhaps because tadpoles remained in contact with one another only briefly.

Structural Observations

Although foam-making clearly involves the spitting and wriggling behaviour of tadpoles, it is possible that there are histological and physiological specialisations associated with this behaviour. To establish this, it was necessary to compare different stages of *L. fuscus* tadpoles and to compare *L. fuscus* tadpoles with those of other relevant species. The structures examined, as most likely to be involved, were the buccal cavity and the skin.

Buccal cavity

Kenny (1969b) has provided a light microscope description of the buccal mucus-secreting glands of tadpoles, and Wassersug (1976) has reviewed the terminology of these structures. There are two sets of glands: a crescentic band of secretory pits at the posterior limit of the buccal roof; and rows of secretory ridges on the ventral surface of the ventral velum. PAS — stained sections of foam-making tadpoles showed that these glands are all fully active (Fig. 1a) and therefore that they are the source of the mucus-bubbles the tadpoles spit out. However, this may not be a specific adaptation, since tadpoles of most species at stage 27-28 would be in water and feeding using their buccal glands to trap food. Is it therefore adaptive for these glands to be functional before entering the water? A useful comparison is with hatchlings of *Colostethus trinitatis*. *C. trinitatis* is a dendrobatid, and males carry tadpoles on their backs from terrestrial nests to water. *C. trinitatis* tadpoles from males backs are at about the same stage as *L. fuscus* foam-making tadpoles, but PAS-stained sections of their buccal cavities showed glands which were much less active (Fig. 1b).

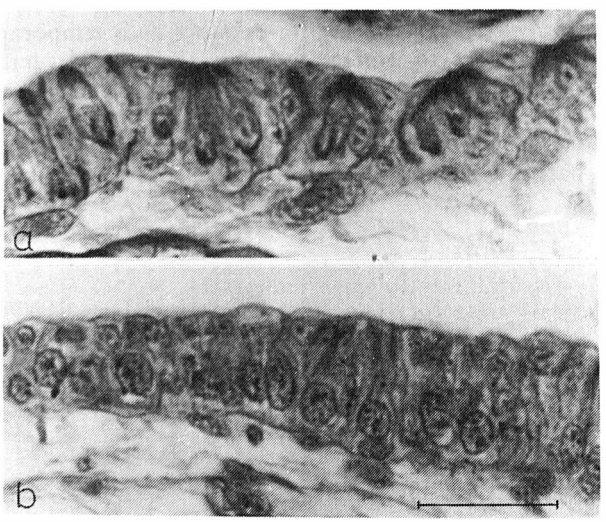

Fig. 1 Secretory pits of the buccal roof, stained PAS and light green. a) *L. fuscus* foam-making tadpole. b) *C. trinitatis* tadpole from an adult male's back. Bar = 20 μm. The dark staining at the apical ends of clusters of gland cells in figure a shows active mucus production. Note the relative lack of staining at the equivalent position in Fig. b.

Skin

Transmission electron microscope sections of foam-making tadpole skin showed numerous mucus secretory droplets, some of them open, lined up at the skin surface, in between microridges. This appearance was not noticeably different from skin of the same

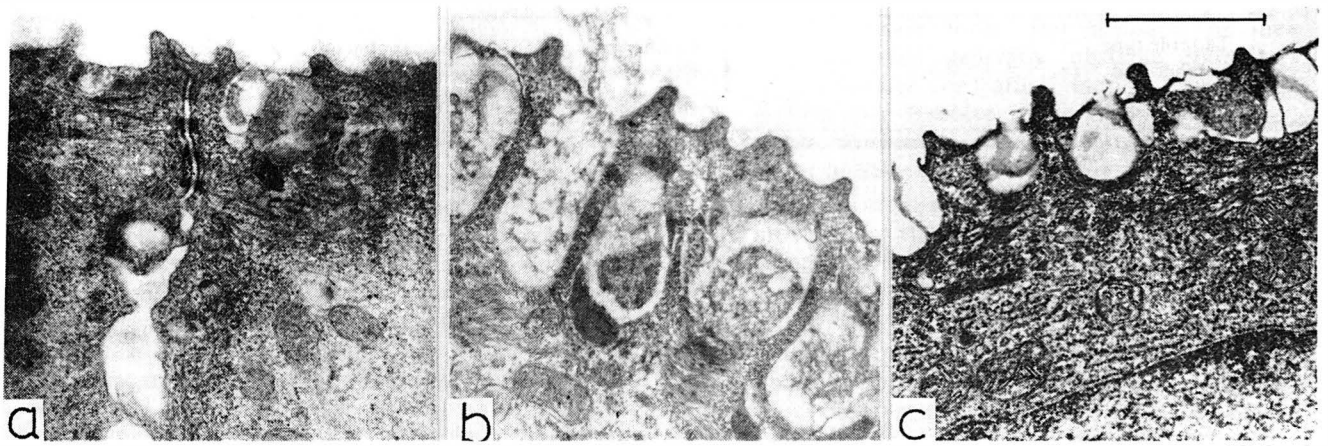

Fig. 2 Transmission electron micrographs of tadpole skin, to show mucus secretory droplets and microridges in section. a) *L. fuscus* foam-making stage tadpole. b) *L. fuscus* stage 32 tadpole. c) *C. trinitatis* tadpole from an adult male's back. Bar = 1 μm.

stage from other species examined, or from skin of later stage *L. fuscus* tadpoles. *C. trinitatis* tadpoles from the male's back again offer a useful comparison: their skin was as well supplied with mucus secretory droplets as that of foam-making *L. fuscus* (Fig. 2).

Scanning electron microscopy showed that epidermal surface microridge patterns varied between species, but also between adjacent cells on the same area of skin (results not shown).

Two other skin structures are worth reporting on. Leydig cells are large diameter cells with a clear watery cytoplasm found in larval amphibian epidermis and located in sub-surface layers. Kelly (1966) suggested, on the basis of experiments with the urodele *Taricha torosa* that Leydig cells act as a temporary water store to protect the skin against short-term desiccation. However, of the tadpole species I looked at, only *Bufo granulosus* had prominent Leydig cells; foam-making tadpoles had very few (Fig. 3). Finally, hatching gland cells, located in the frontal region, are

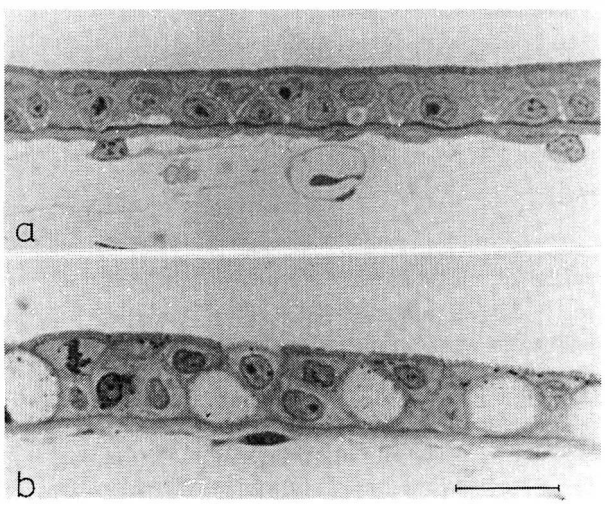

Fig. 3 Toluidine blue stained 1 μm sections of tadpole skin to show Leydig cells — large empty looking cells located beneath the surface of the epidermis. a) *L. fuscus* foam-making tadpole, lacking Leydig cells. b) *B. granulosus* stage 28, with prominent Leydig cells. Bar = 20 μm.

normally active around the time of hatching, and regress over the next few days (Yoshizaki and Katagiri, 1975). Since *L. fuscus* tadpoles do not undergo normal morphological development while in foam, it seemed possible that the hatching gland might not regress, and even that it might have a role in foam production. However, I found that hatching gland regression did occur in foam-making tadpoles, though at a slower rate than in feeding tadpoles. These results will be more fully reported elsewhere.

DISCUSSION

From the behavioural observations made here, it is clear that stage 27-28 *Leptodactylus fuscus* tadpoles make foam primarily by spitting out bubbles from their mouths, and to a lesser extent by the wriggling action of their tails. Wriggling movements are also responsible for breaking larger bubbles into smaller ones, and bursting some bubbles altogether. Since the first bubbles made by newly-washed tadpoles are unstable, whereas later-made ones are longer lasting, it is reasonable to suggest that stability is the result of mucus secreted progressively by the tadpoles the longer they are out of water.

From the evidence, the inability of single tadpoles to make foam is partly due to their lower activity. In groups, tadpoles somehow stimulate each other to be active, probably by being in contact, and the spitting movements are usually associated with overall body wriggling which itself forms some bubbles. It may also be that a single tadpole cannot produce as high a local concentration of bubble-stabilising mucus as a group: this could explain why groups of 10 tadpoles form stable foam more quickly and reliably than groups of three.

Some components of foam-making behaviour were seen in other kinds of tadpoles. *Colostethus trinitatis* tadpoles wriggled more vigorously and more frequently, but they did not stay together, nor did they spit bubbles. *Physalaemus pustulosus* and later stage *L. fuscus* tadpoles initially made spitting movements, wriggled actively and did stay together, but all kinds of movements, particularly spitting soon stopped.

Therefore, though the components of foam-making behaviour are not specific to *L. fuscus* stage 27-28 tadpoles, the overall behavioural repertoire is.

From the histological and ultrastructural evidence, it is likely that the secretion of mucus by *L. fuscus* buccal glands prior to entering water is a specific adaptation to foam-making behaviour. No other feature showed clear evidence of specific adaptation. Mucus secretion by cells all over the skin may certainly help, but occurs generally in tadpoles. Hughes and Wright (1970) have suggested that skin surface microridges help anchor mucus films to skin, though other functions are possible. Since mucus films are as important to tadpoles in water as in foam, there is no reason to suspect that the microridge pattern of foam-making tadpoles should be specifically adapted to this behaviour. Fishelson (1984) has classified microridge patterns in fish epidermis, where they have systematic value, but no similar study has been made on tadpoles, and there is no known functional significance in the pattern variations.

ACKNOWLEDGEMENTS

I wish to thank Professor J. S. Kenny and his colleagues at St Augustine for their help and encouragement; the Carnegie Trust and the University of Glasgow for financial assistance; Helen Hendry for electron microscopy, Pauline Hannan for light microscopy, Patricia Johnston for typing, and Margaret Reilly for commenting on the manuscript.

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