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FUNCTIONS OF THE FOAM IN THE FOAM-NESTING LEPTODACTYLID *PHYSALAEMUS PUSTULOSUS*

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ABSTRACT

The possible functions of floating foam nests in frogs of the family Leptodactylidae were investigated using the common neotropical species *Physalaemus pustulosus* as an example. The results showed that:

1. The foam serves little thermal function, with foam and water temperatures being very similar, and time to hatching in foam compared to water also being similar.
2. The foam has some limited ability to protect eggs and hatchlings from desiccation.
3. Tadpoles of another species attacked intact foam nests, and isolated eggs were palatable to aquatic insect larvae and other tadpole species.

These results are discussed in the context of reproductive mode evolution, and other possible functions of foam — aeration and nutrition — are considered.

INTRODUCTION

It is well known that a common feature of the anuran family Leptodactylidae is the deposition of eggs in a mass of foam, either on the surface of water, or in a burrow. As Martin (1967, 1969) and Heyer (1969) have suggested, the family appears to show an evolutionary shift away from reproduction in water to an increasingly terrestrial mode, culminating in species with larvae that do not feed and are entirely terrestrial. However, the function of the foam, in the presumably primitive species which deposit foam nests in water, often along with other anuran species that show more conventional breeding modes, is not clear. In the literature, floating foam nests have three suggested functions: 1. reduction of predation, 2. prevention of desiccation, and 3. alteration of egg temperatures. These suggestions seem rarely to have been tested, except for temperature where the two published studies given contradictory results.

Using the widely distributed neotropical leptodactylid *Physalaemus pustulosus*, the present study assesses these three possible functions of foam, and speculates on some others.

Physalaemus pustulosus (= *Engystomops*; = *Eupemphix*, Lynch, 1970) is found in Central America, the northern part of South America, and in Trinidad — where my observations were made. In Trinidad, *P. pustulosus* is most abundant at the low altitudes of the Caroni plain, but is also found high in the Northern Range mountains, for example at over 600m in the Arima valley. The frog breeds throughout the rainy season, and following wet nights, its foam nests can be found attached to emergent vegetation at the margins of garden ponds, tyre ruts, flooded grassland or wasteground and even slow-moving drainage ditches (Fig. 1). Nests are made both in shade and in the open, with no obvious preference. The bodies of water used for breeding by *P. pustulosus* are used by many other Trinidadian anurans, but generally lack fish. Each nest contains a few hundred eggs, and tadpoles hatch from the foam 2-3 days after nest deposition. *P. pustulosus* tadpoles are cryptically patterned, bottom-living and not well adapted to life in flowing water. In Panama, *P. pustulosus* is called the tungara frog; in Trinidad, the punglata or pungrara frog (Ryan, 1985; John Seyjagat, personal communication), all names being based on the sound of the call.

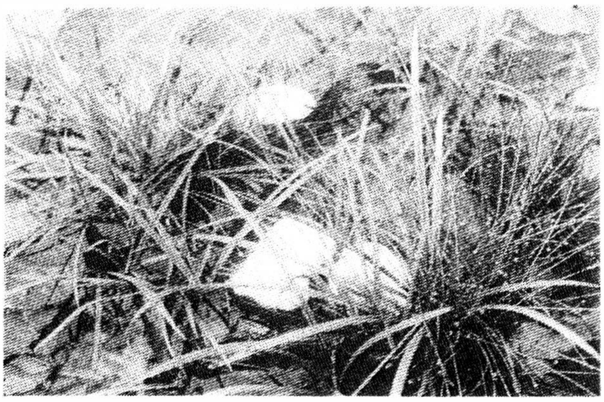

Fig. 1 *Physalaemus pustulosus* nests in typical habitat — a flooded piece of waste ground. Three white foam nests are seen floating at the surface of a shallow pool, amongst vegetation.

MATERIALS AND METHODS

Collection and Nests

The foam nests used in this study were found in temporary pools and drainage ditches near the University of West Indies campus at St. Augustine, on the Caroni plain in Trinidad, during July 1987. Freshly-made nests were collected early in the morning after wet nights. A few eggs were routinely removed for staging to ensure nests were fresh. All eggs tested were gastrulas at the time of collection.

Temperature Measurements and Hatching Time

Five freshly-made nests were placed individually in 2 litre size polythene tubs, and floated on the surface of water at a depth of 18mm. To check on the minimum time to hatching, and on possible effects of high temperatures, 6 eggs were isolated from each nest and floated, essentially foam-free, on the surface of water in a beaker placed in each tub. Two tubs were placed outdoors in the open, two outdoors in shade, and one in the laboratory. The temperatures of water in each tub, air at 5cm above the water surface, the foam surface and foam mass centre were measured at regular intervals over the next 2 days, using an LDC digital thermometer with external sensing probe. Late in incubation, the foam collapsed to a smaller volume and became crusty at the surface if kept dry; when this happened only a single foam temperature measurement could be made. Along with temperature measurements, larval hatching time and the weather were monitored.

Foam Nest Desiccation and Foam Collapse

Nine freshly-made foam nests were placed in 2 litre size polythene tubs, with the foam resting on four layers of damp tissue. Several eggs from each nest were isolated and either floated on the surface of water in a beaker to check hatching time, or placed individually on the surface of the damp tissue beside the foam nest. Nests were then left in the laboratory, either with the tub lid on (6 nests) or with no lid (3 nests). Four more nests were set up, in tubs with no lids, using a layer of damp mud instead of tissue. Egg development was monitored as detailed in the Results section.

To test what happens to the water in the foam, late in incubation, when the foam collapses, four fresh nests were weighed, then incubated floating on water in individual tubs for 48h (when all hatchlings had left the foam) either with the lid on or off. The remaining foam and the total clutch of hatchlings were then weighed.

Observations on Foam Nest Predation

Observations on tadpoles of *Leptodactylus fuscus* attacking complete foam nests were made both in the field and in the laboratory. Since it was unclear whether the tadpoles were feeding on both foam and eggs, eggs were isolated from the foam and placed in tubs for 24h with potential predators — as well as *L. fuscus* tadpoles, odonate nymphs and large tadpoles of *Bufo marinus* and *P. pustulosus*, all collected in the St. Augustine area. Eggs were left floating on the surface of water with a bubble of foam attached, or were sunk.

RESULTS

Temperature Measurements and Hatching Time

Fig. 2 shows the temperature records of the 5 foam nests monitored, as well as the weather during the observation period. Table 1 shows the timetable of hatching of tadpoles from these 5 foam nests.

Nest Number and Location		Time (h) 37	41	45	56	63	69
LABORATORY	Nest		2	20	200	7	
	Beaker		6/6			no more in foam	
OPEN 1	Nest	20	40	50	200	100	All out of foam
	Beaker		1/6	4/6	6/6	a few still in foam	
OPEN 2	Nest	5	40	60	200	5	10
	Beaker		1/6	4/6	6/6	a few still in foam	All out of foam
SHADE 1	Nest			20	30	200	250
	Beaker			3/6	6/6	some still in foam	a few still in foam
SHADE 2	Nest				30	25	200
	Beaker			2/6	5/6*	many still in foam	a few still in foam

TABLE 1: Hatching timetable for foam nests whose temperature changes are recorded in Fig. 2.
The time axis assumes for convenience that nests were made at the middle of the night before they were collected and counts that time as 0h. For foam nests, numbers of hatchlings found in the tubs at each time are recorded. At 56h, foam masses were removed from original tubs so as to obtain an accurate estimate of numbers still to leave the foam. Numbers recorded for 63h and 69h are thereore tadpoles that emerged *after* 56h. Numbers hatching in beakers are given as the proportion out of the original 6 until all are hatched. * = one egg died.

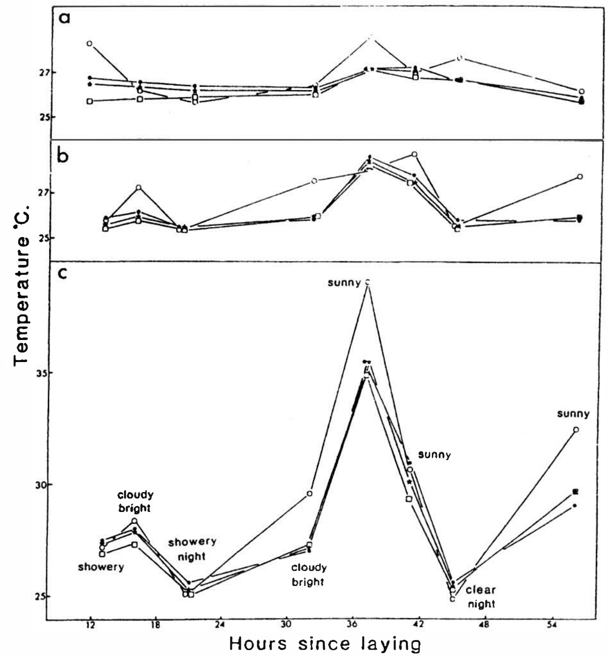

Fig. 2 Temperature records of foam nests. Time is measured since time of laying, assumed as middle of night before nest collection, (a) nest kept in the laboratory, (b) nests in shade outside; each temperature record is the mean of two nests, (c) nests in open outside; each temperature record is the mean of two nests.
Symbols: o = air temperature; ● = water; ⋆ = foam surface; □ = foam centre. Notes on weather at times of recording are given on c.

The results show the following:

1. Air temperature was consistently higher than foam or water.
2. Water temperature was consistently above deep foam, which was usually above foam surface temperature. The differences were small, generally less than 1°C separating all three. The only time when foam temperature was above water was on the final morning, when foam structure had collapsed.
3. In the open, temperature fluctuated considerably, influenced by solar radiation, while in the shade outside and in the laboratory, there was a gentle daily cycle.
4. Hatching time was influenced by temperature. Hatching occurred considerably earlier in the two open outside tubs than in the shaded ones. But short bursts of high temperature are less important than the overall level. Although foam temperature briefly reached 35°C outside in the open, most of the incubation period was spent between 25 and 27°C, not dissimilar to the other tubs. Hatching in the laboratory was not much later than outside in the open, laboratory temperatures being a little above those outside in the shade.
5. There was no evidence for thermal damage to the eggs. Eggs in foam and eggs floating at the water surface without foam outside in the open both exceeded 35°C for a time, and hatched as normal embryos.

Foam Nest Desiccation and Foam Collapse

For the 6 nests incubated on the surface of moist tissue in tubs with the lid on, results were consistent. Isolated eggs on the tissue surface failed to hatch, though they did develop to some extent. The vast majority of eggs in the foam developed and hatched normally. However, this was because these eggs were not subjected to desiccation. With the tub lid on, the foam surface remained moist through a 3 days incubation period. In addition, after about 2 days incubation, the foam structure collapsed, releasing water into the bottom of the tubs: this water was adequate to maintain the tadpoles for the 3 days of the experiment.

In the case of the 3 nests incubated on the surface of moist tissue in tubs with the lid off, the foam surface became dry and crusty after only 1 day and eggs near the foam surface died. When water was added after up to 2 days (2 nests), the majority of tadpoles survived and soon emerged from the foam into the water. But if water was added after 3 days (1 nest), the foam was very dry and there were no survivors. Similarly, there, were no survivors after 3 days in nests on the surface of damp mud in open tubs, though tadpoles had hatched from the foam on to the mud surface.

The amount of water released by foam collapse was measured by weighing nests, then incubating them till the tadpoles had all emerged, and the foam had collapsed, then reweighing the foam. The results are

Nest number and treatment		Initial weight of foam and eggs (g)	Weight of foam after 56h incubation (g)	Weight of hatchlings (g)	Estimated loss of water from foam (g). In brackets % of original foam weight lost
Closed	1	17.8	13.7	2.6	2.1 (13.3%)
	2	14.7	10.1	1.3	3.3 (24.6%)
Open	1	13.9	5.8	0.7	7.4 (56.1%)
	2	13.6	3.6	1.1	8.9 (71.2%)

TABLE 2: Water loss from foam nests incubated in the open, or enclosed. Incubation time is given as hours since laying, estimated as the middle of the night before collection. In calculating water loss, the weight of hatchlings is counted as identical to the initial weight of eggs in the foam.

shown in Table 2. If incubated in the open, water was lost rapidly by evaporation, but if incubated in closed tubs, the nests lost up to a quarter of their original weight by releasing water when the foam structure collapsed.

Egg Predation

On one occasion on the University of West Indies campus, in a temporary pool filled by heavy rain the previous day, I noticed a large number of *Leptodactylus fuscus* tadpoles apparently feeding on the lower side of a newly-made *P. pustulosus* nest. I took this nest and the tadpoles to the laboratory where this behaviour continued in a glass tank.

To make clear whether both eggs and foam were being attacked, isolated eggs were exposed to potential predators, either floating at the water surface, or sunk to the bottom. The results are shown in Table 3. This very preliminary experiment makes clear that *P. pustulosus* eggs are eaten by *L. fuscus* tadpoles, and by odonate nymphs, but apparently not by large tadpoles of their own species.

Predators	Eggs at start	Eggs left after 24h
3 odonate nymphs	15 floating 15 sunk	4 6
6 *L. fuscus* tadpoles	15 floating	0
6 *B. marinus* tadpoles	15 floating	12
12 *P. pustulosus* tadpoles	15 floating 10 sunk	15 10

TABLE 3: Predation of isolated *P. pustulosus* eggs.

DISCUSSION

Being in a foam nest could have several effects on an egg's environment: 1. the egg is removed from water and therefore from any water-borne hazards, such as predators; 2. foam may have helpful physico-chemical properties, related to temperature, gas exchange, desiccation or nutrient supply. The following discussion examines which of these effects occur in *Physalaemus pustulosus* nests, and which could have been important in foam nest evolution.

Temperature

Foam could protect eggs from thermal damage (Gorzula, 1977) or keep them warmer than surrounding water, allowing more rapid development (Dobkin and Gettinger, 1985). My data, more complete than the previous authors, support neither suggestion. Foam and water temperatures were very similar, as were times to hatching in foam and floating on water. Rand (1983) notes that Ryan also was unable to detect any thermal effect of the foam, but gives no details of the methods used, and Ryan (1985) himself does not refer to these observations.

From its distribution and habits, *P. pustulosus* was probably a low altitude species originally, breeding in still or slow-moving water, where overheating seems more plausible as a problem than being too cool. Gorzula (1977) measured the temperature of *Physalaemus enesefae* nests at 14.00h, the hottest part of the day, on the two days following oviposition. He found temperature at the centre of the foam mass almost 5°C less than that of adjacent water at a depth of 1cm, and suggested that foam keeps eggs cool to prevent thermal damage. However, Zweifel (1977) showed that anuran embryos, especially those developing in warm climates, rapidly develop tolerance to short term (2-6h) high temperature exposure. For example, by gastrulation, *Scaphiopus couchii* could tolerate 2h at 40°C, and *Bufo cognatus* 6h at 40.5°C. All species were less tolerant during the earliest stages. Although I did not assess temperature tolerance in *P. pustulosus* eggs, it is noteworthy that gastrulation is reached by the first morning, i.e. before any exposure to the sun, and that the maximum temperatures reported for *P. pustulosus* foam are 34°C (Dobkin and Gettinger), 30.8°C (Gorzula) and 35.5°C (this report): all are well below the maximum tolerable temperatures reported by Zweifel.

Dobkin and Gettinger (1985) did not refer to Gorzula's paper, but got the opposite result. They measured the temperature at the centre of three *P. pustulosus* nests, hourly, during the third day of incubation. One nest was in the open, one in shade and one intermediate. When irradiated by the sun, foam warmed more quickly than the surrounding water, becoming 3°C hotter in the unshaded nest at 13.00h. Later, foam cooled more rapidly than water, so that by mid-afternoon, water was warmer than foam in all three nests. Dobkin and Gettinger were surprised at these results, having expected the white foam to reflect solar radiation and keep eggs cool. They worked at an altitude of 600m (my observations were at less than 50m) which may have made a difference. More important, their observations were all during the third day of incubation, when foam structure has altered, and most tadpoles have hatched: the only time I found foam temperature higher than water was on the morning of the third day.

Desiccation

Since *P. pustulosus* nests are deposited at the margins of pools and ditches, attached to vegetation, they are at risk of drying out if the water level falls before the larvae can hatch. Breder (1946) reported seeing *P. pustulosus* (= *Engystomops*) nests stranded on damp mud above the water level, and I have often seen this too. Furthermore, for species like *Bufo marinus* and *Bufo granulosus* which lay egg strings in the same pools and ditches as *P. pustulosus*, water level falls do kill whole clutches of eggs before hatching. Breder and later authors suggested that *P. pustulosus* foam protects eggs from drying out, but no experimental evidence has been published. My results show that the foam does protect eggs from desiccation during the incubation period, but that if the weather is dry, the foam surface dries out, and protection is very limited.

Hatchlings can also survive in the foam, but only if the foam remains wet — as happens if there are showers. Kenny (1969) reports survival of hatchlings in the foam up to 7 days and Rand (1983) quotes 5 days, but neither states the condition of the foam.

Dead embryos at the surface of drying foam have been reported by Moore (1961) in the Australian *Limnodynastes peroni* whose habits resemble *P. pustulosus*. Since surface drying must be common, it would be adaptive for eggs to be mainly deep in the foam mass, rather than evenly distributed, but I have found no evidence for this.

Release of water by collapsing foam late in incubation seems not to have been reported. The water may provide a temporary pool for hatchlings in stranded nests but only briefly if the weather is dry. Breder (1946) noticed a wet streak leading to water from a stranded nest, which he regarded as 'made by the dissolving froth and serving as a path for the escaping tadpoles'. I have not seen this, but sudden release of water by collapsing foam could work this way.

We need to be careful before suggesting anti-desiccation as a primary function of foam — rather than simply necessary once eggs are in foam — since, primitively, eggs were laid in water. Desiccation could have been a problem if the water bodies used by ancestral forms habitually dried out — or if eggs were attached to vegetation and exposed by falling water levels. Although I have seen entire clutches of bufonid eggs perish in dried up pools used also by *P. pustulosus*, these pools may be atypical, since they must be very hazardous for tadpoles too. Indeed, though some tadpole species can survive several days under rocks and leaves if the pool dries up (Downie, 1984), *Physalaemus pustulosus* tadpoles show little of this ability (Downie, unpublished observations). If slow-moving streams were a common original habitat, eggs would be deposited close to the edge, attached to vegetation to prevent currents carrying them away. In the rainy season, streams rise and fall rapidly, presenting a high risk of exposing such eggs, and a means of avoiding desiccation could have been a major advantage.

Predator Avoidance

Martin (1967), Heyer (1969) and Ryan (1985) have suggested that foam nests offer protection from predation by removing the eggs from water, but it is clear that protection is not absolute. I found that intact foam nests were attacked by tadpoles of *L. fuscus* and that isolated eggs were predated by aquatic insect larvae and by tadpoles. Moreover, Villa *et al.* (1982) showed that a variety of arthropods, particularly 'frogfly' maggots, specifically predate leptodactylid foam nests — though these are 'new' predators and need not affect the argument that foam nests originally evolved to reduce egg predation. A larger scale study is needed to show whether foam nests have any advantages over other egg protection devices, such as egg toxicity.

There have been reports of tadpoles predating the eggs of other anuran species. After observing *L. fuscus* tadpoles attacking *P. pustulosus* nests, I found that Ryan (1985) had seen a similar attack by *Agalychnis callidryas* tadpoles. Banks and Beebee (1987) have reported that predation of *Bufo calamita* eggs by tadpoles of *Rana temporaria* and *Bufo bufo* is a significant factor in competition between these species. This could also be the case for *L. fuscus* and *P. pustulosus*. *L. fuscus* lays eggs in foam nests in burrows beside temporary pool sites, often in advance of rain (Kenny, 1969; Downie, 1984). When the pool fills, *L. fuscus* tadpoles enter and start to feed, but *P. pustulosus* nests are not deposited till rain has fallen, and are therefore available as food to *L. fuscus*.

Other Functions

The temporary pools used by *P. pustulosus* may become hypoxic on hot still days, given their abundance of micro-organisms and the low solubility of oxygen in warm water. Zweifel (1968) has pointed to the advantages of bufonid egg strings and single eggs at the surface film, over large jelly masses, in such conditions. Presumably, being surrounded by a mass of air bubbles might be even better. This idea needs testing, but since foam nests are deposited on nights following rain, which reaerates ditches and puddles, and the incubation period is only 2 days or so, oxygen lack seems more likely to be a problem for tadpoles than for eggs.

Foam could be a food source for hatchlings, either directly or by offering a substrate for micro-organisms. Kenny (1969) noted that hatchlings may stay up to 7 days in the foam but did not comment on whether they grew. Tanaka and Nishihara (1987) have recently checked this possibility, using *Rhacophorus viridis viridis*, which makes terrestrial foam nests at pond edges. Hatchlings reared in water containing foam grew more than those reared in water with no food source. Tanaka and Nishihara concede that their result is preliminary: they have not tested hatchlings kept in the foam nest itself, not do they know what produces the nutrient effect — the foam itself or micro-organisms involved in foam breakdown. In ground-nesting leptodactylids, Pisano and Del Rio (1968) have suggested that nest foam has growth inhibitory functions, but there is no evidence for this in *Physalaemus*.

CONCLUSION

Floating foam nests have been seen (Martin, 1967; Heyer, 1969) as an intermediate stage in the evolution of terrestrial reproduction in the leptodactylids, but they need also to be investigated in their own right. It is surprising, given the abundance, particularly of *P. pustulosus*, how little experimental work has been done on this problem. My results suggest that floating foam does not have important thermal properties, but that anti-desiccation effects do exist. It is also likely that the foam has anti-predator properties, but that this effect is by no means complete.

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