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FUNCTIONS OF THE FOAM IN FOAM-NESTING LEPTODACTYLIDS: THE NEST AS A POST-HATCHING REFUGE IN *PHYSALAEMUS PUSTULOSUS*

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ABSTRACT

At 28°C, isolated *Physalaemus pustulosus* eggs hatch after approximately 40 hours incubation. However, few tadpoles emerge from foam nests at this time. From nests incubated so that the foam remains moist, emergence occurs progressively over the next day. If the foam is allowed to dry on top, complete emergence takes even longer. Manipulation of the incubation environment shows that emergence is not stimulated by dark or light, nor does it occur at a particular time of day. Since hatching occurs at Gosner stage 21 and the last tadpoles to emerge from foam have reached Gosner stage 23-24, it is suggested that late emergence allows hatchlings to continue development to a more advanced stage in a protected environment: the foam acts as a post-hatching refuge. However, it is also shown that tadpoles emerging early are able to grow to Gosner stage 25 by the time the last tadpoles leave the nest: remaining in the nest therefore bears a cost. In addition, part of the delay in emergence may simply result from the time small tadpoles take to wriggle free from a large mass of cohesive foam.

INTRODUCTION

While it is well-known that many members of the anuran family *Leptodactylidae* deposit their eggs in foam nests, either in burrows or floating on the surface of water, it is less clear what the functions of these foam nests are. Possible functions have been suggested by several authors (reviewed by Downie, 1988) but experimental evidence has often been entirely lacking or anecdotal at best: see, for example, Hödl's (1990) interesting but very briefly documented suggestion that foam nests prevent egg predation by conspecific tadpoles. Downie (1988, 1990) investigated a number of possible functions for the floating foam nests of the common neotropical leptodactylid *Physalaemus pustulosus* and found best evidence for foam as a protection against egg predation, mainly by tadpoles of other species. This work considered the foam nest as a container for eggs. However, Kenny (1969) noted that *P. pustulosus* tadpoles (named *Eupemphix pustulosus* in his paper) may remain some days in the foam after hatching and it is therefore possible that the nest has some useful properties for these later stages of development. The purposes of this paper are to document the timing of nest departure by *P. pustulosus* tadpoles, to investigate possible environmental cues for nest departure, and to consider the possible reasons why *P. pustulosus* tadpoles remain in the the nest after hatching.

MATERIALS AND METHODS

COLLECTION OF NESTS

The *Physalaemus pustulosus* foam nests used in this study were found in drainage ditches near the University of the West Indies campus at St Augustine, on the Caroni plain in Trinidad during July and August 1991. Freshly-made nests were collected early in the morning following wet days or nights. Eighteen complete nests collected on four separate days contributed to this study.

NEST, EGG AND TADPOLE INCUBATION

Foam nests were incubated floating on the surface of dechlorinated tapwater. Since incubations were relatively short, and foam is at the water surface, no aeration was necessary. Whole nests were incubated in 2 l rectangular polythene tubs containing 1.5 l water, either with the lid on or off. I cut other nests into pieces so that different treatments could be given to eggs from the same batch, and incubated each piece at the surface of 150 ml water in a 250 ml glass beaker with a plastic petri dish lid. To compare the time of emergence from foam with the time of hatching, I removed 10 eggs from each nest and incubated them singly at the surface of water in 250 ml glass beakers. To allow eggs to float, one or two foam bubbles were kept attached to each egg. Most nests were incubated in a laboratory with artificial light on during the day, but subject to natural lighting at night (in July and August, it is dark by 19.00 h and light again by 06.00 h). This treatment is hereafter termed 'ambient'. Some nests were incubated in this laboratory in constant darkness and some under constant artificial lighting. The laboratory air temperature remained fairly constant at 28-29°C, with the temperature of the water in beakers and tubs about 1°C less. To vary the time of day at which hatching could be expected, I incubated some eggs and nest pieces in an air-conditioned laboratory at an air temperature of 25-26°C and water temperature of 24-25°C, (hereafter termed 'cool' temperature); others were incubated outside in the shade, where the temperature during the middle of the day rose a little over 30°C. After hatching, some tadpoles were grown in 2 l polythene tubs in 1.5 l aerated dechlorinated tap-water with a mud bottom to simulate field conditions. Tadpoles were fed with crumbled tropical fish food flakes.

DETERMINATION OF TADPOLE DEVELOPMENT

To assess the stage of development after different times and treatments, tadpoles were fixed in Bouin's fluid and staged using Gosner's (1960) table. Body lengths (anterior tip to junction of tail and body) were also measured, using an eyepiece graticule in a Wild M5 stereo-microscope, magnification x12.

INFLUENCE OF TIME OF DAY, LIGHT AND TEMPERATURE ON HATCHING AND EMERGENCE FROM FOAM

Five nests, collected on two separate mornings, were used in this experiment. Each was sub-divided into 10 approximately equal pieces (1.5 x 1.5 x 1.0 cm) and each piece floated on water in a beaker. For each nest, I incubated two pieces in

each of five ways: outside, cool temperature, and ambient temperature in the dark, in constant light or with ambient illumination. For each nest and for each treatment, 10 eggs were isolated and incubated as single eggs floating on the surface of water. Numbers hatching (as single eggs) and entering water (from floating foam) were counted at intervals. To assess whether the onset of darkness or daylight acted as a stimulus for hatching or entering water, I recorded numbers at 18.00 h (an hour before dark), 20.00 h - 21.00 h (just after dark) and at 06.00 h (dawn).

Influence of Nest Size and State of Hydration on Emergence From Foam

To assess whether complete nests showed the same hatching and emergence pattern as cut-up pieces, I incubated 10 whole nests in 2 l tubs under normal lighting and temperature conditions. Six of these were incubated with the lid on (where the foam surface remains moist) and four with the lid off (foam surface becomes dry as the upper part of the nest dehydrates) to assess whether dehydration is a factor in tadpole emergence. Ten single eggs were withdrawn from each nest and incubated in beakers to determine the time of hatching.

Assessment of Loss of Growth When Tadpoles Remain in Foam

In this experiment, I incubated four complete nests in 2 l tubs with the lid off (this maximises the time spent in foam - see Results) in ambient conditions of temperature and lighting. Once more than five tadpoles had emerged into the water, a few were fixed for staging and the others transferred to tubs for feeding. Once all tadpoles had emerged from a particular nest, a sample of the last emergers was fixed, and also those early emergers which had been allowed to grow.

RESULTS

Influence of Time of Day, Light and Temperature on Hatching and Emergence From Foam

The times of hatching and emergence from foam for tadpoles from five different sub-divided nests collected on two separate mornings and incubated under different conditions are given in Fig. 1. For ease of comparison the data are plotted as if all eggs were fertilised at midnight during the night before collection. In practice, it is likely that the time of fertilization differed for the different nests: hatching was consistently earlier in nest 1 than in 2 and 3, and a little later in 4 and 5. The data are presented for individual nests, rather than giving mean values, because of this variability and because the demands of other field work made it impossible to count hatchlings at precisely the same times of day for the two different batches of nests.

Batches of single floating eggs hatched, under all conditions, over a relatively short period. At 'ambient' temperature and lighting, no hatchlings were seen at 33 h, but all had hatched by 42 h. For nests 4 and 5, all hatched between 38 and 42 h. An ANOVA was carried out, using the time to hatching of each floating egg as the dependent variable, to assess the differences between incubation in ambient conditions, constant light and constant dark. This gave $F_{2,147} = 5.77$ with $0.01 > P > 0.001$. There was a significant difference between eggs incubated in constant light (mean time to hatching ±SD, 40.1 ± 2.5 h) and those in constant darkness (41.5 ± 2.0) but neither of these treatments was different from the ambient group (40.9 ± 1.8). However, I should point out that hatching time was only measured as those hatched after 2-4 hourly intervals, rather than the precise hatching time. It is possible that this method has masked variability in the data, thereby producing a spurious statistical difference. It would be worthwhile to repeat this experiment to collect finer resolution data.

Incubation outside depended on temperature: nests 1 and 2 were incubated outside on a hot day, and hatching occurred a little earlier than normal; nests 3, 4 and 5 were incubated on a cooler cloudy day and hatching occurred at the same times as in the laboratory. Cooler incubation conditions, not surprisingly, delayed hatching. Of the 'cool' group, nests 1 and 2 were kept at 23.5-24°C. till 54 h, then transferred to ambient conditions: hatching occurred between 45 and 58 h. Nests 3, 4 and 5 were kept at the cool temperature only till 38 h: as a result, hatching was completed earlier, by 54.5 h.

Emergence from foam consistently occurred over a much longer time-span than hatching from single eggs. Commonly, one or two tadpoles emerged from foam at the same time as single eggs hatched, but most tadpoles emerged much later. At ambient temperature, the interval between the observation of 100% hatching of single eggs and 100% emergence from foam was 17±1.9 hours (n=8, mean ±SD.). Constant darkness and constant light had no consistent effect on the pattern of emergence. Although most emergence at 'ambient' temperature occurred during the hours of darkness, there was no evidence that darkness acted as a stimulus for emergence. Counts made at 20.00 h or 21.00 h, soon after sunset, showed no surge in emergence compared to the time before sunset. Neither did dawn (06.00 h) mark any change in the rate of emergence. In support of this conclusion of a lack of a dark-light effect, incubation of nests 3, 4 and 5 at 23.5-24.0°C till 38 h achieved an approximate 12 h delay in emergence, with the majority of tadpoles emerging during daylight hours.

Emergence From Complete Foam Nests and an Effect of Dehydration

Since it was possible that the subdivision of complete nests could affect the emergence pattern, two sets of complete nests were incubated, one covered, the other open, and the numbers of tadpoles emerging counted at intervals. The 'covered' treatment simulates nests in wet conditions when the foam remains moist at the surface. The 'open' treatment simulates dry conditions when the foam becomes dry and crusty at the surface: both commonly occur in the field. Sets of single eggs were removed from some of these to assess hatching time. The results are shown in Fig. 2. It is clear that tadpoles remained in the complete nests for some time after the hatching of single eggs, just as occurred in sub-divided nests. However, the data suggest that tadpoles remained somewhat longer in complete nests than in sub-divided ones, and longer in dehydrated nests (incubated in the open) than in moist ones (incubated in closed containers). Since only some nests were followed to completion, comparisons need to be made at earlier stages. At 58 h incubation, subdivided nests at 'ambient' temperature and lighting showed a mean emergence of 87.7%, whereas complete nests by the same time showed 45.6% with lids on and only 5.6% with lids off. A Students t test performed on arcsin-transformed percentages for complete nests showed that the difference between lid-on and

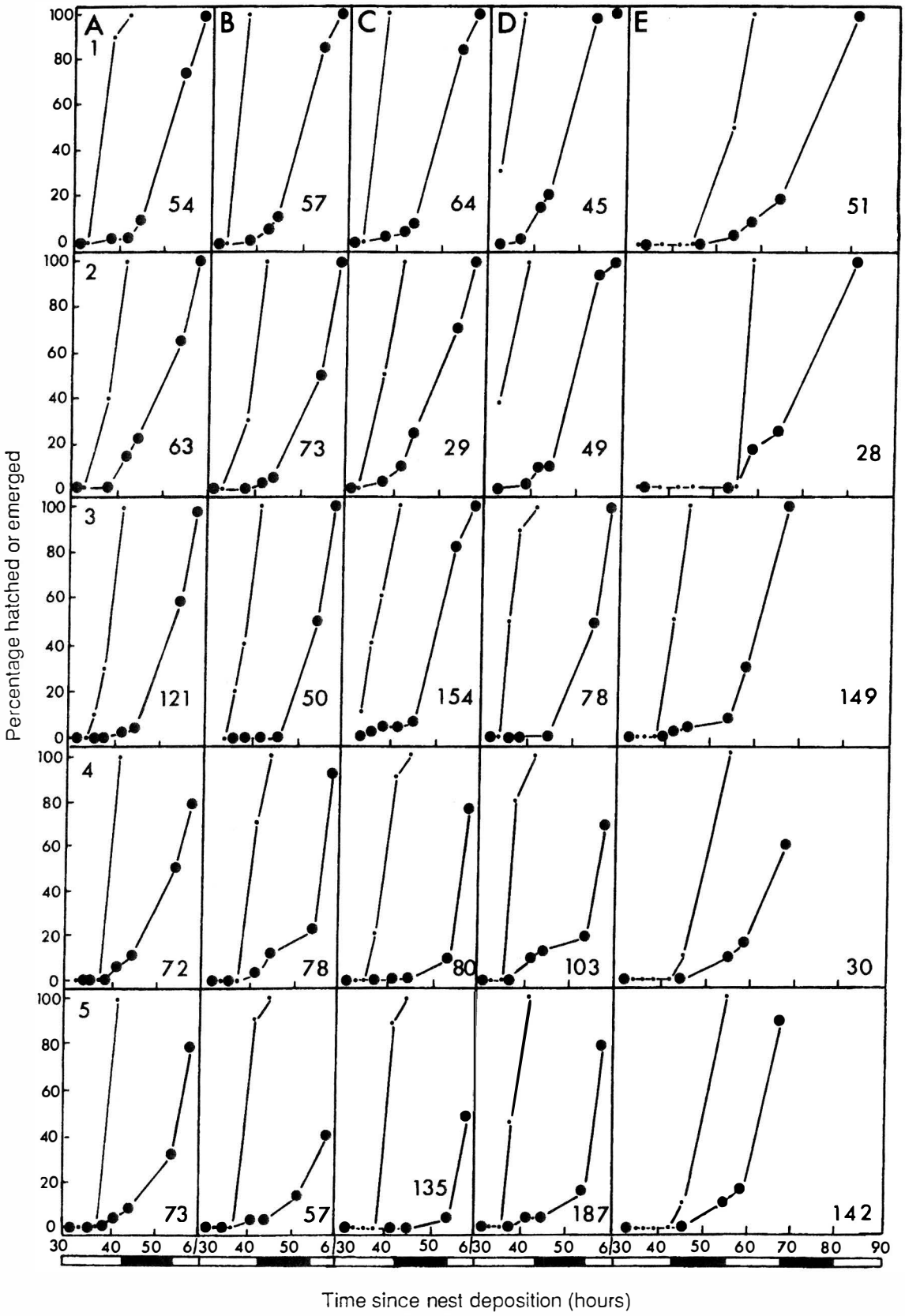

FIG. 1 Hatching and emergence from subdivided nests incubated in different conditions. Numbers hatched or emerged are given as percentages of the total number. Time is measured since a standardised time of nest deposition (see text). Conditions are A - normal temperature and lighting; B - normal temperature, constant darkness; C - normal temperature, constant light; D - incubated outside; E incubated in cooled laboratory part of time (see text). There are samples from five (numbers 1-5) nests for each condition. Small dots show hatching from groups of single eggs. Large dots show emergence from pieces of foam. Figures at the bottom right of each box denote the total number of tadpoles emerging from foam at each treatment. The bar at the bottom of the figure denotes hours of daylight (clear) and darkness (black).

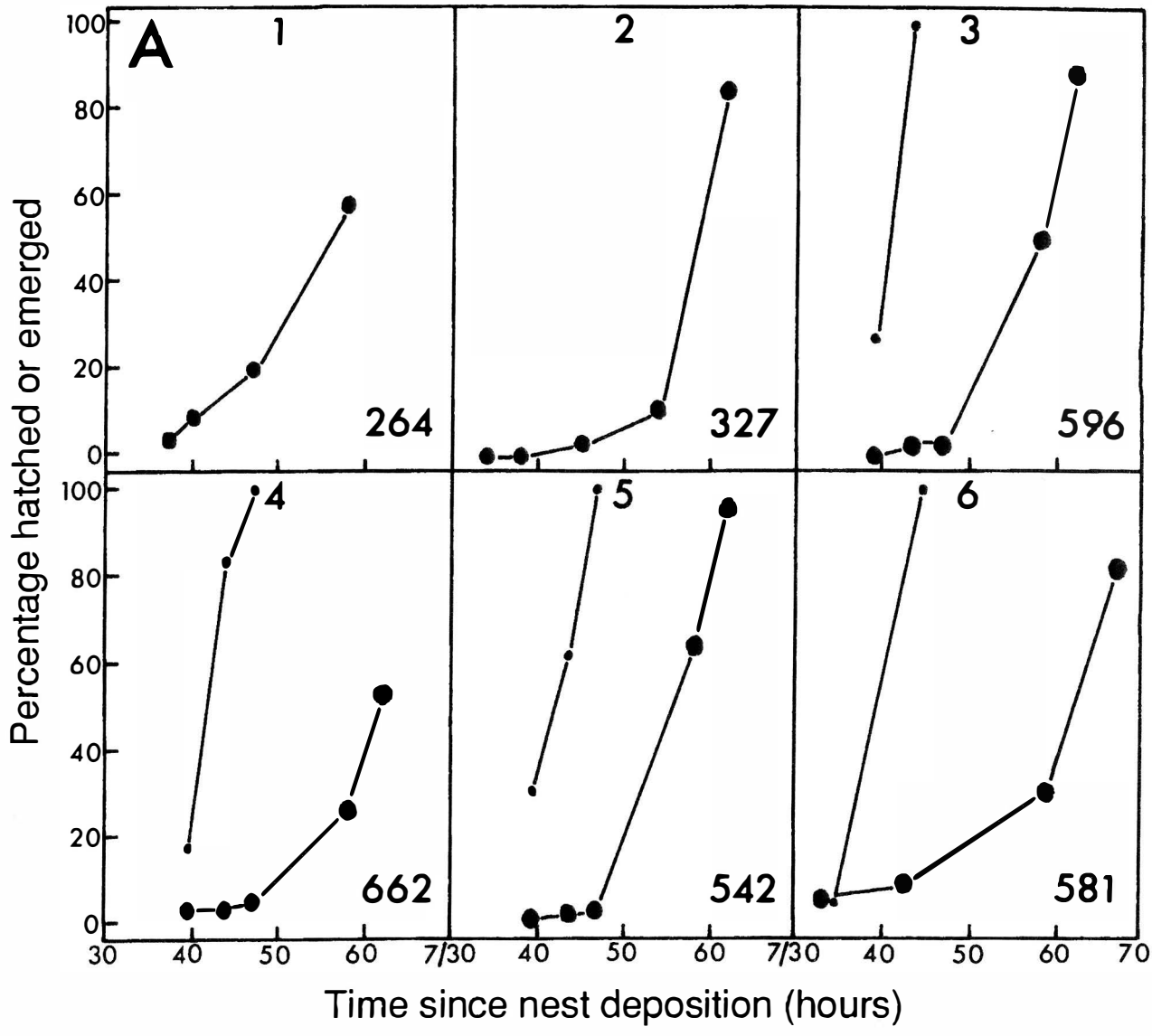

FIG. 2 (Above and opposite) Hatching and emergence from complete nests, incubated with lid on (nests A 1-6) or lid off (nest B 1-8). Numbers hatched or emerged are given as percentages of the total number. Time is measured since a standardised time of nest deposition. Small dots show hatching from groups of single eggs (not carried out for all nests). Large dots show emergence from complete nests. Figures at the bottom right of each box denote the total number of tadpoles emerging from each nest.

lid-off treatments was highly significant ($P < 0.001$). For complete nests, the moist-dehydrated difference was maintained at 67 h: 81% emergence with the lid on and only 36.6% with the lid off ($0.05 > P > 0.02$ in this case).

Post-Hatching Behaviour, Development and Growth

As shown in Fig. 1, single eggs at 'normal' temperature hatched at around 38 h after foam deposition. Examination of recently hatched tadpoles showed them to be at Gosner stage 21 whereas the best developed embryos still in their vitelline membranes were at stage 20. After hatching, tadpoles tended to remain motionless for some time, attached to the bottom or sides of the container by their adhesive glands.

One possible explanation for the difference in time between hatching from single eggs and emergence from complete nests could be that the single eggs were the first in a clutch to be fertilised. This is unlikely, given that the single eggs were taken randomly from the nests and hatched over a 4 h period, whereas emergence from foam extended over a much longer period. Another explanation could be that somehow, single eggs floating at the water surface develop faster than those in foam. This possibility was investigated by allowing tadpoles hatched from single eggs to continue developing, without food, until all tadpoles had emerged from floating foam nests. Samples of the last emergers and earliest hatchers were then fixed and compared. A similar comparison was made between the earliest and later emergers from complete foam nests, incubated floating on the surface of water in closed tubs. The results of both tests are shown in Table 1.

It is clear from these results that in terms of morphological developmental staging, there was no detectable difference between early hatchers and emergers, and late emergers, though there were small size differences. Students *t*-tests were performed on the size differences for each nest separately since a nest effect was evident. Numbers were too small to test differences between tadpoles hatching from single floating eggs and

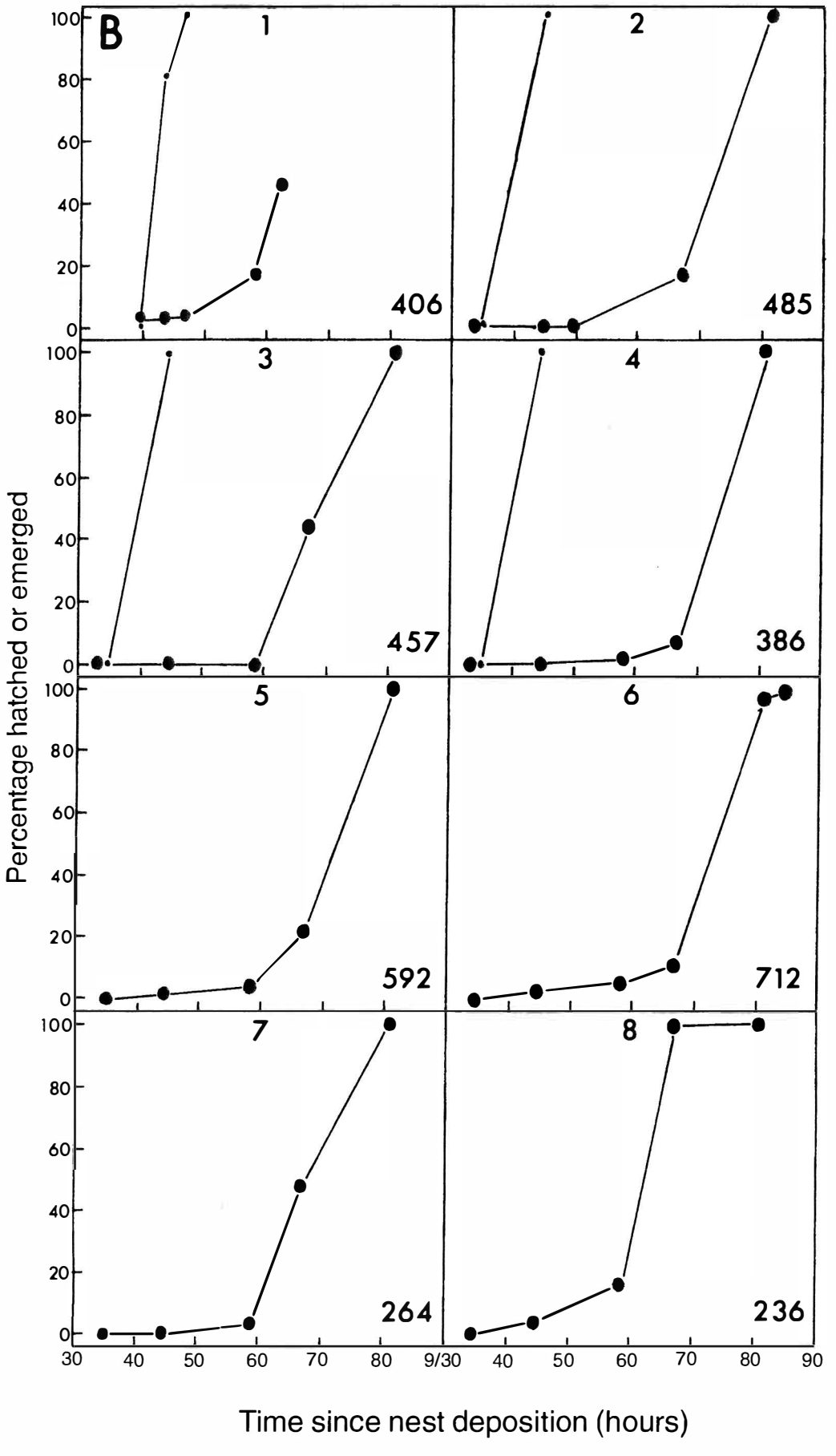

FIG. 2. Continued (previous page and above).

(a) Tadpoles hatched from single floating eggs then kept in water without feeding, compared to latest emergers from floating foam. All measurements soon after time of latest emergence.

		Nest 1	Nest 2
Single eggs:	hatching time (h)	42	42
	number measured	5	10
	Gosner stage	23-24	23-24
	mean body length (mm)	2.82	2.84
	(± SD)	(± 0.07)	(± 0.15)
Floating foam:	emergence time	63.5	63.5
	number measured	2	7
	Gosner stage	23-24	23-24
	mean body length (mm)	2.50	2.75
	(± SD)	(± 0.24)	(± 0.08)

(b) Tadpoles developed in complete foam nests: early emergers compared to latest emergers. All measurements soon after time of last emergence.

		Nest 1	Nest 2	Nest 3	Nest 4
Early emergers:	emergence time (h)	54	54	43.5	43.5
	number measured	14	13	4	9
	Gosner stage	23	23	23	23
	mean body length (mm)	2.53	2.56	2.29	2.17
	(± SD)	(± 0.1)	(± 0.12)	(± 0.29)	(± 0.12)
Late emergers:	emergence time (h)	59.5	59.5	62	62
	number measured	10	15	13	11
	Gosner stage	23	23	23	23
	mean body length (mm)	2.37	2.46	2.13	2.05
	(± SD)	(± 0.08)	(± 0.1)	(± 0.18)	(± 0.08)
	P	<0.001	0.05>*P*>0.02	NS>0.1	0.02>*P*>0.01

TABLE 1. Comparison of morphological stages and body sizes of tadpoles entering water early with those entering late.

		Nest 1	Nest 2	Nest 3	Nest 4
(a) Early emergers:	Number	26	24	7	10
(characters at time	Time (h)	58.8	44.5	58.5	44.5
of emergence)	Gosner stage	22	21-22	22	21-22
	mean body length (mm)	2.17	not clearly	2.08	not clearly
	(± SD)	(± 0.14)	defined		defined
	number measured	3	0	1	0
(b) Early emergers:	Number	23	21	6	8
(characters after	Time (h)	84	84	84	84
growth to time of	Gosner stage	25	24	24-25	25
late emergers)	mean body length (mm)	3.00	2.57	3.17	3.05
	(± SD)	(± 0.1)	(± 0.23)	(± 0.07)	(± 0.17)
	number measured	10	10	4	5
(c) Late emergers:	Number	463	19	136	224
	Time (h)	81	84	81	81
	Gosner stage	23-24	23	24	23-24
	mean body length (mm)	2.59	2.46	2.70	2.66
	(± SD)	(± 0.7)	(± 0.2)	(± 0.08)	(± 0.21)
	number measured	9	16	12	7
	P (*b* v. *c*)	< 0.001	NS>0.2	< 0.001	0.01>*P*>0.001

TABLE 2. Comparison of morphological stages and body sizes of tadpoles entering water early and allowed to grow with those entering late.

those hatching last from floating foam (Table 1a). However, at the same time of fixation, tadpoles emerging early from complete foam nests were significantly longer than those emerging late in three out of the four nests measured (Table 1b). In the remaining nest, there were only four early emergers to measure. Another relevant observation is that in floating foam nests past the stage where hatching has occurred, hatched tadpoles could be seen at the upper surface of the foam, often wriggling around actively. These were not simply hatchlings from eggs which happened to be at the foam surface, since there were normally rather few such eggs, yet many tadpoles were found at the surface. This observation was made only in foam nests where the foam surface was moist. When nests were incubated in open tubs, the foam surface dried out, and tadpoles were not seen at the upper surface.

It is possible that tadpoles that emerge early from nests have some advantage in gaining access to food before those that emerge late. This was tested by isolating groups of early emergers from complete nests, then allowing them to grow in conditions similar to those they would meet in the field in tubs with water over a muddy bottom, with a little food added. These were grown until the time the latest emergers appeared from each nest. Samples of fed and late emerging tadpoles were then fixed for comparison. The results are shown in Table 2. In all cases, tadpoles that emerged early and were given access to food grew and developed so that they were in advance of those that emerged last, though in one case (nest 2), the difference was very small. A Student *t*-test was performed on the body length results for the fed and late emerging tadpoles from each nest separately. The larger size of the fed tadpoles was significant in three out of the four nests measured. In the remaining nest, early fed emergers failed to grow and were no larger than later emergers at the time of fixation.

DISCUSSION

This study began with the hypothesis that emergence from foam might be synchronised as a predator-satiation device. This clearly does not happen in the case of *Physalaemus*. At the ambient temperature used here (28-29°C), eggs hatched into the foam around 38 h after foam deposition: a few emerged from the nest soon afterwards, but it took many hours for all to emerge, the actual time depending on the size of the piece of foam and on whether the foam surface remained moist or became dry. Kenny (1969) reported that hatching into the foam took 72 h and that tadpoles remained there up to seven days. Unfortunately, Kenny did not give data on incubation temperatures: his times are about twice those reported here.

What are the reasons for the delay in tadpole emergence? There are a number of possible explanations, some adaptive, others not. First, the sequence of emergence might simply reflect individual differences in development rate. The evidence is against this explanation. Randomly chosen isolated eggs hatched over a period of as little as 2 h, yet the delay in emergence was 16 h or more. Unfed early emergers were at the same developmental stage as the latest emergers when the latter left the nest. Since Hödl (1990) found that complete nest construction in the related *Physalaemus ephippifer* took only 40 mins, with egg release occurring over only part of that time, there can be little variation in time of fertilization in a complete batch of eggs. My results do show a small difference in body size between unfed early emergers and newly emerged late emergers, but this may simply be due to more complete hydration of the tissue.

Next, the tadpoles might choose a particular time of day to emerge: for example, since many aquatic predators, such as odonate larvae, are primarily visual, there could be an advantage to emerging in the dark. In salmonids, where hatchlings remain many days in gravel nests before emerging, it is well established that emergence is linked to the onset of darkness, with a high proportion emerging in the first dark hour (Brannas, 1987). However, the evidence gives no support to this idea in the case of *P. pustulosus*. Tadpole emergence showed no response to constant dark or light, or to changes in natural light. When low temperature slowed development by approximately 12 h over the normal period to emergence, tadpoles emerged during daylight rather than in the 'normal' darkness.

A different adaptive explanation is to see the foam nest as a protective refuge. Hatching stage larvae may be particularly vulnerable. It may therefore be advantageous to remain some time in the foam after hatching: despite the lack of food, development can continue based on the remaining yolk reserves. Individual tadpoles may then make the choice either to emerge or to remain longer in the nest, retaining the protection but suffering a potential delay in growth. This explanation has no particular evidence against it. Previous work (Downie, 1988; 1990) has shown that foam nests offer effective protection to eggs against predators: they can clearly do the same for hatchlings. Larval development does continue, without additional food, in those that stay in the nest, from Gosner stages 21 to 24. The gradual pattern of emergence fits the idea of tadpoles choosing between protection and potential growth. The ability of early emergers to develop when fed beyond the stage of later emergers shows that there is a real cost in late emergence. Although the delay in emergence and the amount of growth achieved in the first day may both seem small, *P. pustulosus* tadpoles can reach metamorphosis in 2 weeks but live in temporary pools that have a high risk of drying up (Downie, unpublished): in the circumstances, an emergence delay of even one day is significant. What is lacking so far is a demonstration that early emergers (stage 21-22) are more vulnerable to predation than late emergers (stage 24) but this is at least plausible given the maturation of the locomotory and other systems that occurs over this period.

Though this explanation is attractive, a final more trivial possibility must be examined. A *Physalaemus* foam nest is quite large (of the order of 80 cm^3 in volume) and the foam is a highly cohesive material. It may simply be that hatchlings find it difficult to make their way out of the nest and that complete emergence therefore takes time. The following evidence suggests that this is at least part of the explanation for the emergence delay. Total emergence from sub-divided nests is quicker than from complete ones. Drying out of the nest, which makes the foam more cohesive, delays complete emergence. However, this seems unlikely to be the entire explanation. Eggs are distributed throughout the foam, some very close to the bottom and therefore to water, yet very few emerge just at the time of hatching. Finally, the observation that many tadpoles move to the top rather than the bottom of the foam suggests that they have some reason to stay there.

It is not known how common delayed emergence is in amphibians, though it is well known in fish (Brannas, 1987) where hatching is often followed by a long period where the young fish grow using their yolk reserves in the protected environment of a nest (for example, salmonids) or egg case (for example, dogfish). In anurans, delayed emergence only seems a possibility where relatively large eggs are deposited in some sort of protective nest. In the *Leptodactylus* '*fuscus*' species group, tadpoles remain up to several weeks after hatching in nests laid on land in burrows (Downie, 1984). However, where eggs are laid in water singly or in jelly strings as in *Xenopus* and the bufonids, rupture of the outer jelly capsules occurs before hatching from the vitelline membrane (Duellman & Trueb, 1986; personal observation on *Bufo granulosus*): in these species, there is no potential for the delayed emergence seen in *Physalaemus*

The recent study of Magnusson & Hero (1991) demonstrates the importance of anuran egg predation by aquatic invertebrate larvae and by tadpoles. The present study suggests the need to investigate predation pressure on early post-hatching stages too.

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REFERENCES

Brannas, E. (1987). Influence of photoperiod and temperature on hatching and emergence of Baltic salmon (*Salmo salar* L.). *Canadian Journal of Zoology* **65**, 1503-1508.

Downie, J. R. (1984). How *Leptodactylus fuscus* tadpoles make foam, and why. *Copeia* **1984,** 778-780.

Downie, J. R. (1988). Functions of the foam in the foam-nesting Leptodactylid *Physalaemus pustulosus*. *Herpetological Journal* **1**, 302-307.

Downie, J. R. (1990). Functions of the foam in foam-nesting Leptodactylids: anti-predator effects of *Physalaemus pustulosus* foam. *Herpetological Journal* **1**, 501-503.

Duellman, W. E. & Trueb, L. (1986). *Biology of Amphibians* New York: McGraw-Hill.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.

Hödl, W. (1990). An analysis of foam nest construction in the neotropical frog *Physalaemus ephippifer* (Leptodactylidae). *Copeia* **1990**, 547-554.

Kenny, J. S. (1969). The Amphibia of Trinidad. *Studies of the Fauna of Curacao and other Caribbean Islands* **29**, 1-78.

Magnusson, W. E. & Hero, J. -M. (1991). Predation and the evolution of complex oviposition behaviour in Amazon rainforest frogs. *Oecologia* **86**, 310-318.