Dedicated to the memory of Professor Peter R. Bacon

Comparative Breeding Ecology of the Frogs *Leptodactylus fuscus* and *Physalaemus pustulosus* in Trinidad, West Indies

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ABSTRACT

Foam nest numbers deposited by two leptodactylid frogs *Leptodactylus fuscus* and *Physalaemus pustulosus* were monitored at five ephemeral ponds in the village of Lopinot over a 40 day period in the rainy season. *L. fuscus* laid more nests after dry or light rainfall days, and none after very wet days; *P. pustulosus* tended to lay more nests after wet days, and fewer after light rainfall days. *L.fuscus* nest burrows were positioned so as to be inundated by water only after very wet days. Water levels in the ponds fluctuated markedly, with one pond drying out on four separate occasions during the study. Although many more *P. pustulosus* nests and total egg numbers were deposited than *L. fuscus* (228,300 *pustulosus* eggs estimated compared to 6300 *fuscus*), numbers of tadpoles of the two species found in the ponds were fairly similar. The different reproductive strategies of the two frogs, and their suitability for ephemeral pond breeding, are discussed in the light of these results.

INTRODUCTION

Leptodactylus fuscus and *Physalaemus pustulosus* are two of Trinidad and Tobago's commonest frogs, with widespread distributions on both islands (Murphy 1997; Kenny, 1969 named these frogs *L. sibilatrix* and *Eupemphix pustulosus*). *L. fuscus* (the whistling frog) and *P. pustulosus* (the pung-la-la frog) both owe their local names to their calls and are found predominantly at lower elevations on agricultural land and urban fringes, whenever choked ditches, deep tyre-ruts and temporary pools occur.

Both species are foam-nesting leptodactylids: *L.fuscus* nests are made in burrows in the banks of ditches and pools and are hard to locate, because the parent frogs seal the burrows after completing the foam nest. Each nest contains around 100 large (2.0 mm diameter) pigmentless eggs which hatch after about 2 days incubation and then remain in the foam nest from a few days to several weeks, depending on the occurrence of heavy rain to flood the burrow, allowing the larvae to enter the pool as feeding stage tadpoles (Downie 1994). *P. pustulosus* nests are made on the surface of water, usually at the edges of pools and ditches. They are conspicuous and white, containing about 300 small (1.5 mm diameter) pigmentless eggs which hatch after about two days incubation and soon drop from the nest into the water below; after 2 days further development, they are feeding stage tadpoles (Downie 1988 and 1993).

L. fuscus and *P. pustulosus* commonly breed in the same pools in Trinidad. The aim of this study was to compare the success of their different breeding strategies in relation to the characteristics of their habitat.

STUDY SITE AND METHODS

The study site was a set of five temporary pools in and around the grave yard at Lopinot Village in Trinidad's Nothern Range. We visited the site 20 times, at two day intervals, between 1100 and 1500 h each time, between 12 July and 22 August, 1999, and have also made observations at this site in earlier years and since then. The pools are rain-fed and serve to water cattle which graze the graveyard: in 2003, the pools were mainly filled in, possibly to reduce mosquito breeding. Figure 1 illustrates the lay-out of the site as it was in 1999. At the first visit, pond dimensions and basic characteristics were assessed. At each visit, the following were noted:

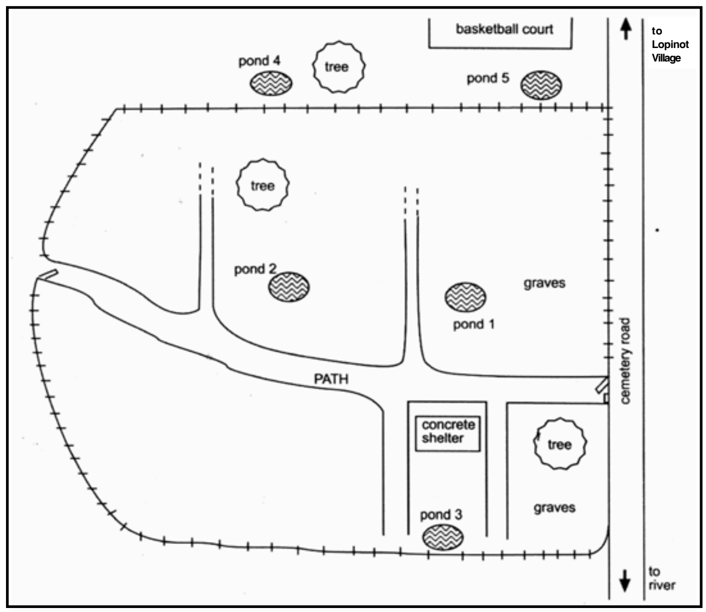

Fig. 1. Map of the graveyard site at Lopinot (not scaled)

- Pond depth at the deepest point, to 0.5 cm using a metre stick.
- Rainfall at the site since the previous visit, using a rain gauge left in an open but secluded position.
- Water temperature to 0.1 °C using a digital thermometer placed at the centre of the pond.
- Water pH using pH paper.
- Freshly made *P. pustulosus* and *L. fuscus* nests.

It was straightforward to count *P. pustulosus* nests, except that mating pairs are sometimes so close together that their nests fuse: we estimated the number of individual nests in a fused foam mass from size and shape. We located *L. fuscus* nests by systematically inserting the handle of a tablespoon into the mud surrounding each pond to a distance of about 30 cm from the outer edge: this method located empty frog holes, hatched nests containing larvae and fresh foam nests. The height of each nest above the water level was recorded by means of a metre stick and spirit level. A combination of this measurement and pool depth at the same time allowed calculation of the position of each nest relative to the bottom of the pool.

In addition, every second visit (10 in total), we used a small aquarium net to sample the relative proportions of the two tadpole species and any predators; the length of each pool was swept twice, close to the bottom, and all tadpoles and insect larvae caught were returned to our laboratory for identification and counting.

During this study, a large tethered water buffalo was moved around the site to graze the grass, and it often spent time sitting in the nearest pond. We did not think it advisable to disturb this animal: this led to lack of data from each of the ponds on 1-2 occasions during the study.

RESULTS

Nest numbers and rainfall

Table 1 shows the number of nests recorded for each species related to the rainfall recorded for the period since the previous visit. A Spearman's rank correlation showed a significant positive relationship between number of *P. pustulosus* nests and rainfall ($r_s = 0.715$, $P < 0.05$) but a significant negative relationship between *L. fuscus* nests and rainfall ($r_s = -0.803$, $P < 0.05$). The most anomalous result in this pattern was the large number of *P. pustulosus* nests for the 0.0 mm rainfall record. This was our first sample and, although no rainfall was recorded on the previous days, it followed a period of very heavy rainfall which had filled the pools. Given the concealed nature of *L. fuscus* nests, it is likely that we underestimated their numbers, but we feel that our technique was systematic enough to ensure that under-counting was not serious. Since we sampled every second day, and hatching occurs after two days, any discovery of nests that were later than two days post-hatching indicated nests missed on previous sample days: their numbers were low.

Table 1. Numbers of nests of *Physalaemus pustulosus* and *Leptodactylus fuscus* found on each sample day, arranged according to rainfall during the previous two days.

Rainfall (mm)	Number of nests	
	P. pustulosus	*L. fuscus*
0.0	61	14
0.5	25	2
2.5	26	5
4.0	17	4
8.0	26	12
9.0	46	3
12.0	31	4
13.0	35	3
13.5	39	5
14.5	36	5
18.0	52	0
20.0	55	3
21.0	52	1
24.0	68	2
49.0	43	0
60.0	51	0
66.0	53	0
109.0	49	0

Pond choice and characteristics

Table 2 shows the characteristics of each pond, and the number of nests of each species recorded during the study period. Ponds 1-3 showed good numbers of both species nests, with *P. pustulosus* predominant; pond 4, the coolest pond with least temperature variation, due to shading by a large tree, contained the smallest numbers of nests; pond 5 had the largest number of *P. pustulosus* nests, but no *L. fuscus* at all (a result supported by finding no *L. fuscus* tadpoles). There was no obvious relationship between nest numbers and pond dimensions or temperature, other than that the only cool pond had fewer nests than the others. The lack of relationship with pond dimensions may not be surprising, given that the ponds were quite similar in size. There was also no relationship with water pH, consistently recorded as around pH 5.5.

Table 2. Characteristics and nest numbers of the different ponds.

Pond	Temperature ∞ C mean ± SD	Mean dimensions (m: length x width x depth	Total *P. pustulosus* nests	Total L. fuscus nests
1	32.0 ± 4.5	2.9 x 2.3 x 0.19	133	18
2	32.0 ± 4.4	2.4 x 2.3 x 0.17	151	24
3	34.7 ± 4.6	2.1 x 1.3 x 0.13	108	14
4	26.2 ± 1.3	1.7 x 1.4 x 0.14	92	7
5	32.4 ± 4.0	2.6 x 2.5 x 0.19	277	0

Pond drying and community composition

Water depth profiles for the five ponds are shown in Figure 2. Broadly, these followed the same pattern, related to rainfall, but the

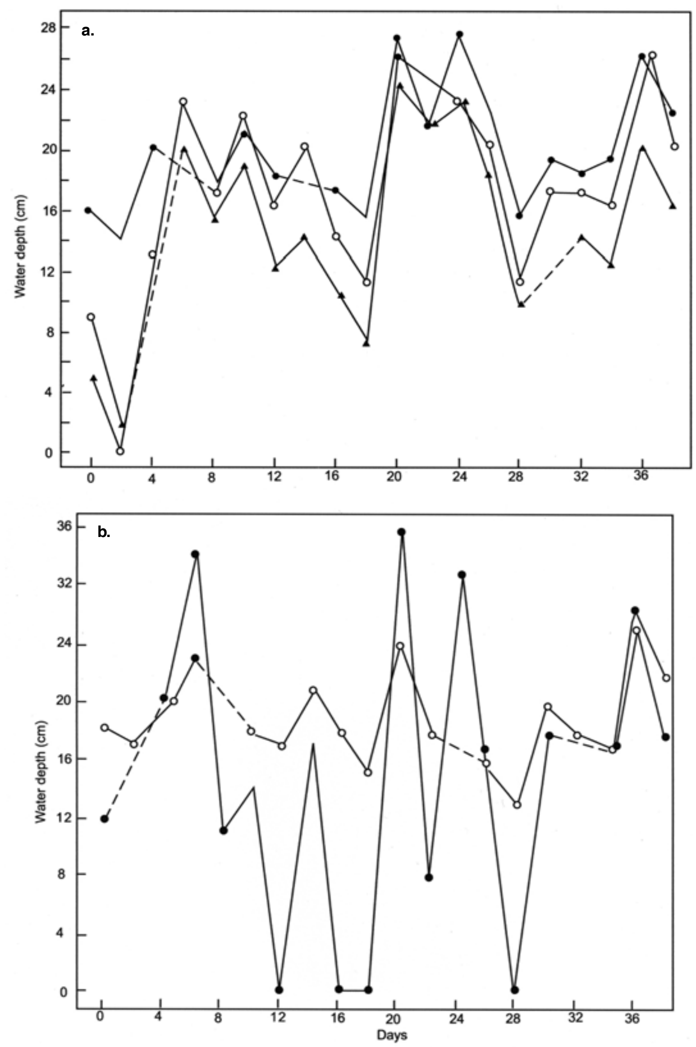

Fig. 2. Water depth changes in the five Lopinot ponds a) ponds 1-3; b) ponds 4-5.

different physical characteristics of different ponds did lead to important consequences for tadpoles. Pond 4 was less basin shaped than the others and dried up most often (dry on four occasions). There was also most probably a relationship between pond depth and water temperature (likely since a small depth of water heats up faster than deep water), though we did not have enough data to discount complicating effects like degree of cloud cover: however on two days when water depth was zero in the shaded pond 4 and low in the others, water temperature was close to or in excess of 40°C.

Table 3 shows the mean numbers of *L. fuscus* and *P. pustulosus* tadpoles captured from the different ponds. The data support the earlier result of a lack of *L. fuscus* in pond 5. Notice that despite their being many more *P. pustulosus* nests overall than *L. fuscus* (Table 2) and the fact that each *P. pustulosus* nest contains three times as many eggs as an *L. fuscus* nest, the numbers of tadpoles of the two species were surprisingly similar. The sampling nets we used had a mesh size of about 1mm^2 since finer nets would have taken too much of the sediment from near the bottom of the ponds. Recently hatched *P. pustulosus* located at the surface of the mud would not be taken by this method. Our sampling therefore estimated only tadpoles that had succeeded in growing well past the start of feeding stage. Another feature is that the only pond where the recorded numbers of *L. fuscus* tadpole clearly exceeded *P. pustulosus* was pond 4, the only pond to dry up more than once during the study.

Table 3. Mean numbers of tadpoles taken from the different ponds (9-10 samples through the study).

Pond	*P. pustulosus*	*L. fuscus*
1	31.0	21.0
2	26.9	22.4
3	14.2	16.4
4	7.9	16.0
5	51.3	0

The number of predators, nearly all odonate nymphs, that we captured was quite small, only three for every hundred tadpoles. However, this was certainly an underestimate of actual numbers since most of the nymphs sit in the mud at the bottom of the pond.

At pond 2, we found six foam nests of *L. validus*, another of Trinidad's leptodactylids, on one occasion, but did not see tadpoles of this species during later visits. Other frog species have been known to breed at the site in other years, including *Hyla crepitans*, *Phyllomedusa trinitatis* and *Bufo marinus*, but they did not occur in 1999: we are therefore essentially dealing with a two species comparison.

L. fuscus nest distribution

Table 4 shows the height distribution of *L. fuscus* nests at each pond, in comparison with water depth variation and the measured high water mark. Nests occurred over quite a narrow range of heights, well up the sides of the pools, but within high water mark.

Table 4. Position of *L. fuscus* nests in relation to pond depth and high water mark in the different ponds (no *L. fuscus* in Pond 5).

Pond	Number of nests found	Nest height (cm: mean ± SD)	Pond depth (cm: mean ± SD)	High water mark (cm)
1	18	20.7 ± 1.7	19.7 ± 4.1	27.5
2	24	21.1 ± 4.3	16.8 ± 6.3	26.0
3	14	19.6 ± 2.2	14.5 ± 6.3	24.0
4	7	21.7 ± 2.2	14.8 ± 10.8	32.0

DISCUSSION

The results presented here compare reproduction in two tropical leptodactylid frogs at one location in Trinidad. The study was carried out in July and August, in the midst of Trinidad's rainy season (May-December approximately) and, although we have sampled only a part of the season, it is clear that reproduction in these frogs is very different from the pattern characteristic of temperate region species, which tend to breed only once in early spring. Breeding in the tropics tends to occur throughout the rainy season, whenever conditions are suitable. Several factors may be responsible for this difference: higher temperatures in the tropics may allow the tadpole phase to be relatively short, providing time for several successive batches of young to complete metamorphosis in a season; another factor may be the unpredictability of the rains: if temporary pools have a significant risk of drying out before tadpoles can reach metamorphosis, a bet-hedging strategy where individuals reproduce several times over the season is likely to be more successful than a single reproductive event (Philippi and Seger 1989).

We do not know how often individuals mate over the season. This could be established in *P. pustulosus* by marking the frogs in a population and following a breeding season. Previous work on this species (Ryan 1985; Green 1990) has concentrated on reproductive activity in males, but the number of foam nests produced by a population over a season must be related to the ability of females to produce multiple batches of eggs. We know of no relevant field study, but Davidson and Hough (1969) found that females of *Physalaemus* (= *Engystomops*) *pustulosus* in a laboratory population kept under rainy season conditions were able to ovulate every 4-5 weeks. *L. fuscus* would be harder to study because of the secretive nature of these frogs: males call from their burrows and become silent when observers come close; finding and marking a population would be very difficult.

We also do not know how long it takes for a newly fertilised egg to grow into a tadpole at the start of metamorphosis: in all amphibians, development time is affected by temperature, but also by environmental factors such as nutrient availability and competition, both intra- and inter-specific. In a laboratory study (Downie, Miller and Langhorne, unpublished data) we have found that at 28°C, well fed *P. pustulosus* and *L. fuscus* at low density can reach metamorphosis after 17 and 14 days respectively, compared to Kenny's (1969) report of 6 weeks for both species. Kenny did not give details of rearing conditions but we suspect his tadpoles were kept at high density. Increases in density and growing the two species together both lead to increases in development time in our experience. Laboratory data of this sort can only provide a general indication of what may be happening in the field, where the situation is complicated by diurnal temperature fluctuations, alterations in density due to water volume changes, the entry of new batches of tadpoles and the effects of predation and food availability. However, they can establish the minimum time needed to complete development, since other factors lead to increases.

Of the ponds in our study, pond 4 dried out three times and pond 2 once during the 38 day period we observed them. Given that we only visited every second day, it is also possible that other ponds may have dried temporarily, especially pond 3 (Figure 2a). This shows that drying out within the period of development from egg to metamorphosis (minimum 14-17 days at 28°C in the laboratory: all our ponds except the shaded pond 4 had higher mean temperatures, but they were all measured when at their hottest, around the middle of the day) is a likely occurrence. If this happens, *L. fuscus* tadpoles have a chance of survival for some time: Downie (1984) and Downie and Smith (2003) have shown that *L. fuscus* tadpoles can survive several days out of water on a damp substrate (they may hide under leaves at the bottom of a dried up pond), but *P. pustulosus* die very quickly in these conditions. Previous work on pond drying in relation to tadpole growth has often demonstrated a speeding up of tadpole maturation (e.g. Crump 1989), but these studies have generally started with single egg clutches and a simulation of drying as the tadpoles near metamorphosis. The multiple ovipositions and unpredictable filling and drying of the ponds seen in our study clearly provide a much more complex situation.

The positions of *L. fuscus* nest burrows, all 20-22 cm above the pond bottom, suggest that they are situated so as to be inundated (allowing the tadpoles to emerge) only after very heavy rainfall. During our study, this occurred only at days 6, 20, 24 and 36. As noted earlier, *L. fuscus* hatchlings are able to survive several weeks in their foam nests, when they are in a state of suspended development (Downie 1994). The advantages of this strategy are twofold: emerging into the pool only after very heavy rain increases the likelihood that there will be enough water to complete development; also, being already at the feeding tadpole stage shortens overall development time and allows these tadpoles to prey on the eggs of frogs which reproduce only after rain, such as *P. pustulosus* (Downie 1990). Our data on nesting in relation to rainfall shows that *L. fuscus* did not breed on very wet days, whereas lowest numbers of *P. pustulosus* nests occurred after dry days: our results were not able to make this entirely clear partly because we sampled every second day, not daily, and because our study period was generally quite wet. Lack of breeding by *L. fuscus* on very wet days could be for two reasons: inundated mud may be unsuitable for making burrows; and it may be disadvantageous for *L. fuscus* nests to open before the larvae have got to the feeding stage. We suspect that nest burrow position may be related to the characteristics of the pond. In a previous study (Downie and Barron, unpublished data) of a ditch at Lopinot, *L. fuscus* nests were mainly about 10 cm above the ditch bottom, but this was a shallow ditch along which water could flow quickly: again, it only reached nest inundation depth occasionally. How *L. fuscus* can assess the best place to make burrows is not clear.

So far, the breeding strategy of *L. fuscus* seems much better suited to the characteristics of the site than *P. pustulosus*, yet *P. pustulosus* is present breeding in large numbers. How can this be explained? An extra factor is the frogflies whose maggots can destroy whole batches of *L. fuscus* eggs (Downie *et al.* 1995) though their frequency of occurrence is not high enough to be a serious limitation. In Trinidad, frogflies do not seem to attack *P. pustulosus* nests, though *Physalaemus* species are attacked in South America (Menin and Giaretta 2003). It is possible that *P. pustulosus* has some advantageous features that we have been unable to assess, such as better predator avoidance. In a study of tadpole distribution and competition in ephemeral ponds in Texas, Dayton and Fitzgerald (2001) concluded that pond duration and tadpole predator avoidance behaviour helped explain the differential distribution of species. Another factor may be differences in pollution toleration: we did notice that pond 5 where *P. pustulosus* bred abundantly and *L. fuscus* not at all, seemed to suffer more than the others from organic pollution from the water buffalo. The other obvious feature is the sheer difference in numbers of eggs produced by the two species: at an average of 300 eggs per nest, 228,300 *P. pustulosus* eggs were deposited over our study period, compared to only 6300 for *L. fuscus*. This disparity came down to very little difference in tadpole numbers sampled: broadly, therefore, *P. pustulosus* is an r-selected species, reproducing often, with large numbers of small eggs; *L. fuscus* is more k-selected, with smaller numbers of eggs, each of which is larger.

Previous work on these species has emphasised a number of other factors affecting breeding success. *P. pustulosus*, in particular, is a much studied species, *L. fuscus* much less so. Marsh *et al.* (1999, 2000) found that *P. pustulosus* males range between ponds at a scale of 200m apart but that females can only exercise mate choice (by assessing male call quality) at a scale of about 10m. The further apart ponds are, the higher the degree of site fidelity shown by males. Our ponds were about 20m apart and it would be of interest to assess levels of migration and site fidelity.

In another foam-nesting species, *Pleurodema borelli*, Halloy and Fiano (2000) found that oviposition was inhibited by existing high densities of conspecific tadpoles: late stage tadpoles ate new eggs and recent hatchings. We saw no evidence of such an intra-specific effect, though, as noted above, *L. fuscus* tadpoles have been observed to feed on *P. pustulosus* eggs (Downie 1990).

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