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Nesting in the Gladiator Frog, *Hypsiboas boans* (Anura: Hylidae), in Trinidad and Tobago

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ABSTRACT

Nests of the gladiator frog, *Hypsiboas boans* (Anura: Hylidae), were monitored in a tributary of the Caura River, north Trinidad, during the wet season (July 2013) and during the following dry season (March 2014). Most nests were found at the stream edge, in easy communication with flowing water. Nests were either small basins excavated in gravel/sand with a mat of eggs floating on the surface of the contained shallow pool of water or were in vegetation-bound inlets out of the current, with eggs present but with no evidence of an excavation. Excavated and non-excavated "nests" occurred in similar frequencies. Mean clutch sizes of wet season nests were 1078 ± 170 SD (n = 6), whereas those of dry season nests were 1070 ± 271 (n = 6). Even when hatchlings could reach the stream easily, they remained in the nest until seven days after oviposition. One excavated nest was more than 2 m from the stream. During the three-week monitoring period, there were no heavy rains, and the larvae remained in the nest with little sign of growth or progressive development. *Hypsiboas boans* is widely distributed in South America, but these nesting details from Trinidad are different enough from those of South American populations to suggest some local adaptation.

Key words: reproduction, development, local adaptations.

INTRODUCTION

Murphy and Downie (2012) discussed our increasing knowledge of the amphibian and reptile fauna of Trinidad and Tobago, noting that both recent migrations and new methods of analysis add to the islands' species list. Some of Trinidad and Tobago's species are endemics, but many are regarded as belonging to wide ranging species often extending far into mainland South America. However, even where there is substantial evidence that a widely occurring taxon should be considered to be a single species, there may be local adaptations in a population that distinguish it from other populations. From a conservation viewpoint, such local adaptations need to be investigated and understood.

The giant treefrog, *Hypsiboas boans* (Anura: Hylidae), is one of these wide ranging species. It belongs to the *semi-lineatus* group of "gladiator" frogs (named from the aggressiveness of the males and their possession of a prepollical spine used in fighting). The species occurs from the lower Amazon Basin north to Panama and the Guianas (Faivovich *et al.* 2005; Frost 2014) and is widely distributed in Trinidad, inhabiting trees bordering streams and rivers; it does not occur in Tobago.

Kenny (1969) reported that *H. boans* spawns during the dry season (December to April), with the female excavating a nest basin up to 45cm in diameter in gravel or sand on the banks of streams. The basin then fills with water, and the female lays eggs as a floating mat on the surface. Kenny apparently erred in identifying the female as the nest maker; all other accounts refer to the male as nest maker (Murphy 1997; Burger *et al.* 2002; Caldwell 1992; Magnusson *et al.* 1999). Kenny (1969) noted that it was not clear how the hatchlings eventually left the nest. He speculated that this may have happened following showers which, even in the dry season, may have raised the water level high enough to breach the basin walls and allowed the tadpoles to access the stream.

In other parts of *H. boans*'*s* range, nesting behaviour can be variable. Burger *et al.* (2002) reported nests in Peru very similar to those described by Kenny. However, Caldwell (1992) and Magnusson *et al.* (1999) found that *H. boans* sometimes constructed basins but that they could also lay eggs in secluded, still water sites without making a construction, an example of plasticity in nesting behaviour. In this paper, we report on observations of *H. boans* nests in Trinidad during July, well outside the usual nesting season, and we compare them with observations of dry season nests.

METHODS

Genera

The principal location for these observations was an 80m stretch of a tributary of the Caura River (Figure 1: GPS co-ordinates 10° 40, 881′ N, 61° 21, 782′ W, ele-

vation 90.9m) on the southern side of Trinidad's Northern Range. The first set of observations was made each morning from 6-19 July, 2013 and once more on 26 July, 2013. On each visit, the edges of the stream were checked for *H. boans* nests, and the developmental progress of tadpoles in previously located nests was noted. One sample of newly deposited spawn (about 50 eggs) was taken to the laboratory (University of the West Indies, St. Augustine, Trinidad) and incubated in about 1 litre of aerated dechlorinated tap water at ambient temperature to monitor the rate of development. Samples of hatched larvae were taken from two nests in the stream, euthanised by use of a 0.01% aqueous solution of benzocaine and then fixed in formalin to allow accurate measurements to be taken. Larval lengths were later measured by use of a Wild dissecting microscope with a calibrated eyepiece. Clutch sizes were counted in five of the nests on the first or second day after oviposition. We also found and counted one clutch by the side of the Lopinot River (beside the Cemetery Road Bridge). To compare observations made during the wet season (in July 2013) with those made of dry season nests, one of us (MG) revisited the Caura site in March and early April 2014. No nests were found at the original principal location, but careful searching further upstream revealed several recently deposited egg clutches.

Fig. 1. The authors searching for *H. boans* nests at the edges of the Caura River tributary.

Lehtinen (2014) reported nest construction by another species of gladiator frog, *H. crepitans,* in Tobago, a behaviour for this species previously reported only from South America. *Hypsiboas crepitans* also occurs in Trinidad; we have found it breeding in blocked drainage ditches in the Caura Valley but not in rivers (JRD, unpublished observations.). The colour pattern of *H. boans* tadpoles is distinctively different from those of *H. crepitans* (Kenny 1969). The tadpoles that we found in the Caura stream were unmistakably those of *H. boans*.

Description of principal site

The stretch of the Caura River tributary studied is no more than 4m wide and is well shaded by trees and other vegetation. It includes some pools, some shallows with gravel bottoms, and abundant rocks. There are no steep descents on the stretch. During the period studied, despite being in what is normally the wet season, only a few short showers occurred and the water level remained fairly constant, with evidence of a significant rise on only one occasion, during which we observed detritus (plastic waste) deposited at the level of one of the monitored *H. boans* nests (Figure 2). The stream contains abundant guppies (*Poecilia reticulata)* and Hart's rivulus (*Anablepsoides hartii*); burrows in the banks indicated the presence of freshwater crabs.

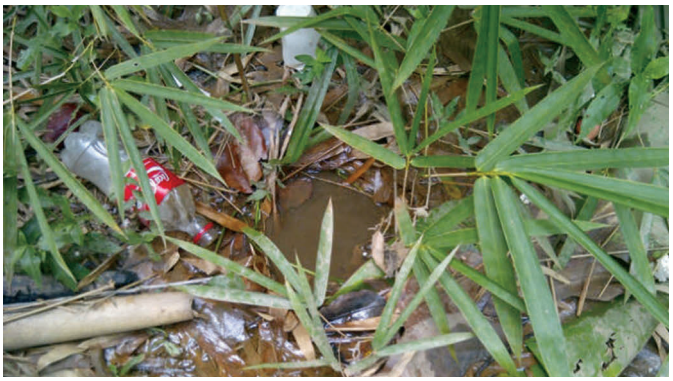

Fig. 2. Small basin nest after water level rise that brought down detritus such as plastic bottles.

RESULTS

Rate of development

The developmental progress of the egg clutch monitored in the laboratory is shown in Table 1. The data here allow assessment of the approximate time of deposition of clutches found in the field (there may be small temperature-related differences between field and laboratory-incubated eggs).

Table 1. *Hypsiboas boans* early development: egg deposition assumed the middle of the night preceding first observation. Stages according to Gosner (1960).

Time after deposition (d)	Developmental stage
1.5	Neurulae, stage 14
2.8	Around hatching, stage 19
3.5	Small external gills, stage 20
4.5	Maximal external gills, stage 23
5.5	Gills fully resorbed, stage 25, mean total length 10.3mm
6.5	Stage 25, mean total length 11.1mm

Clutch sizes and nest descriptions

Data on the nests observed in July 2013 are shown in Table 2. The mean clutch size of those that we counted was 1078 ± 170 SD. Only five of the nests were obviously constructed depressions, with diameters 12-15cm; the remaining five clutches were floating on the surface of the water in natural secluded backwaters; most were contiguous with or very close to the stream side – only one was at a significant distance (more than 2m) away.

At the Lopinot River, as well as finding one nest we found abundant *H. boans* larvae at a wide range of sizes/stages in the shallows at the stream margin. On our first visit to the Caura stream (6/7/13), we found no larvae, only four nests. All but one of the nests we found at Caura were in a short stretch (about 10m) of stream with shallow gravel edges and vegetation- or rock-bounded inlets; downstream of this stretch, the stream was narrower, deeper, and faster flowing. We found only one nest there, in a vegetation-bound small inlet.

Over our Caura monitoring period 6-26 July, 2013, there was no rain heavy and prolonged enough to raise the water level sufficiently to reach Caura nest 1, located more than 2m from the stream side. We sampled hatchlings from this nest regularly over the monitoring period; sizes and stages are shown in Table 3. These larvae would have reached the 11mm stage (Table 1) on 11 July, 2013. Remaining in the nest, they grew slightly over the next few days but then remained at essentially the same length until last sampled on 26 July, 2013, three weeks after oviposition. Development, as assessed by gut coiling, progressed a little over that time.

Observations on the morning of 10 July, 2013 showed that recent rain had raised the stream level enough to reach Caura nests 2-4, all close to the stream. However, many tadpoles remained in all three nests, even the most advanced one (Caura nest 2) with larvae 12.4 ± 0.7mm (n = 7) in length. By the next day, this nest was empty, although nests 3 and 4 still contained many larvae. By the following day (12 July, 2013), nests 3 and 4 still contained some larvae, but many had dispersed around the shallows of the inlet where these nests were located, and some could be found 2-3m downstream. Larvae sampled

Table 2. Clutch sizes and nest descriptions; dates given are for the approximate night of laying back-calculated from stage of embryos upon discovery.

Location, date	Clutch size	Nest description
Lopinot, 4/7/13	940	Shallow water-filled depression in gravel, about 15cm diameter, about 10cm from stream edge.
Caura 1, 4/7/13	1300	Shallow water-filled depression in gravel, about 12cm diameter, 2.5m from stream, separated from stream by a slightly elevated gravel bank.
Caura 2, 3/7/13	870	Depression in sand/gravel, 12cm diameter at stream edge, water at margin of depression contiguous with stream.
Caura 3, 4/7/13	1224	Eggs laid at edge of stream in a backwater; no sign of constructed basin.
Caura 4, 2/7/13	not counted	Shallow water-filled basin, about 12cm diameter, leaves and gravel/ sand base, 40cm from stream edge.
Caura 5, 13/7/13	not counted	Eggs laid at edge of stream at base of large rock in a backwater; no sign of a constructed basin.
Caura 6, 13/7/13	1140	Eggs laid at surface of water at stream edge in an inlet well shaded by vegetation; not a constructed depression.
Caura 7, 13/7/13	994	Eggs laid at surface of water in a partly isolated puddle well shaded by vegetation, about 12cm diameter, well downstream from nests 1-6.
Caura 8, 14/7/13	not counted	Eggs laid in a constructed basin, about 13cm diameter, with a clear lip at the stream edge, made in a sand bank at the stream edge (Figure 3).
Caura 9, 23/7/13	not counted	Eggs amongst a group of rocks in mid-stream; not a constructed basin.

a little way downstream from nests 2-4 on 13 July, 2013 were 12.3 ± 0.8mm (n = 9). By 14 July, 2013, very few larvae remained in nests 3 and 4, and tadpoles could be found as far as 30m downstream from these nests.

Fig. 3. Basin nest with lip of sand and gravel, constructed at edge of large rock.

Table 3. Sizes and stages of larvae remaining in Caura nest 1, 12 to 26 July, 2013. No development past Gosner stage 25. Gut coil development assessed using Nieuwkoop and Faber's (1975) system for *Xenopus laevis*: stages 45-47 would all be classed as Gosner stage 25 (McDiarmid and Altig 1999).

Date	Mean length in MM. ± SD (sample size in parenthesis)	Stage: gut coil development
12/7/13	12.1 ± 0.4 (9)	Stage 45
13/7/13	12.4 ± 0.2 (9)	Stage 45
15/7/13	12.9 ± 0.3 (10)	Stage 46
17/7/13	12.5 ± 0.4 (10)	Stage 46
19/7/13	12.8 ± 0.4 (10)	Stage 46+
26/7/13	12.4 ± 0.3 (8)	Stage 47

On 15 July, 2013, larvae sampled from the stream were 14.7 ± 1.6mm (n = 9), excluding one that was close to metamorphosis and which may have come from further upstream. On 19 July, 2013, after some rain, the water level in the stream allowed hatchlings in nests 5 and 8 (now at day 6 and 5, respectively) to access the stream, but each nest still contained large numbers of larvae.

To assess whether our wet season observations were exceptional, we observed dry season (March and April 2014) nests close to the same principal location in the Caura Valley. Of six nests found, three were in vegetation-bound inlets at the stream margin, with no obvious excavation; the other three also were at the stream edge, but depressions had been excavated in the gravel. Mean clutch size was 1070 ± 271 SD, similar to those of the wet season nests counted in July 2013.

Since we visited the Caura site only during the day, we were unable to observe nest construction and egg deposition. We observed no nest attendance by adults.

DISCUSSION

The main conclusions to be drawn from these observations are:

1. In Trinidad, *H. boans* is variable in nesting behaviour, depositing its eggs in suitable shallow inlets adjacent to the stream as frequently as it excavates a basin for the eggs. Eggs were deposited in inlets where the vegetation reached to the stream edge and where there was no gravel bank, but there was no lack of gravel banks along the stretch of river that we monitored.
2. If a nest is located at some distance from the stream, the hatchlings have to remain in that nest until the stream level rises sufficiently to reach them; during that time, they grow and develop very little. We found no evidence of any parental action to assist them in accessing the stream.
3. Even when the hatchlings are able to access the stream, they remain in the nest until they reach about 12mm long, approximately seven days after egg deposition.
4. We found no evidence of seasonal variation in clutch size or in nest construction (whether excavated or not).

Plasticity of nesting behaviour in *H. boans,* either basin construction or egg deposition in a secluded spot at the stream margin, has been reported by Caldwell (1992) and Magnusson *et al.* (1999), both from Brazil. Caldwell reported basin construction for 12 of 21 nests found, and Magnusson *et al.* reported basin construction for six of 16 nests/sites found. In contrast, Burger *et al.* (2002) found basin construction as the norm (59 of 60) in Peru. The dimensions of basins in Brazil and Peru were substantially larger than those we found in Trinidad (12-15 cm): mean diameters of 36.2cm (Caldwell 1992) and 34 cm (Burger *et al.* 2002) respectively. In addition, clutch sizes in the South American populations were substantially higher than those in Trinidad (means of 1078 and 1070 in our two samples): means of 2531 (n = 4) in Brazil (Magnusson *et al.* 1999) and 1980 (n = 47) in Peru (Burger *et al.* 2002).

Magnusson *et al.* (1999) noted that hatchlings remained in basins for at least a week, with some larger ones lingering for two weeks, and that all had dispersed by three weeks; this implies that larvae develop in these basins. Caldwell (1992) found that larger tadpoles in basins predate smaller conspecifics. Our findings were quite different: hatchlings remained in the nest till about seven

days after oviposition but then dispersed if they could. Tadpoles in nests isolated from the stream remained in the nests but stopped growing. Delayed emergence from the nest may provide an advantage in that the late emerging individuals are more advanced in their development and better able to survive than are early emerging individuals, as found for *Physalaemus* (=*Engystomops*) *pustulosus* emergence from foam nests (Downie 1993). However, it seems unlikely that tadpoles in very isolated nests have any advantage, other than in cases of heavy rains just after eggs have been deposited, when eggs closer to the stream may be washed downstream and die.

The nest and clutch sizes we report for Trinidad are very different from those reported for Brazil and Peru, possibly implying some local adaptation. Our findings that dry season nests were not different from rainy season nests accords with a lack of seasonal variation in clutch sizes in the Tobago glass frog (Lehtinen *et al.* 2014).

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