RESEARCH ARTICLE

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The behaviour of recently hatched Tobago glass frog tadpoles

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ABSTRACT - Egg clutches of the Tobago glass frog *Hyalinobatrachium orientale tobagoense* were collected from streams around the north-east of Tobago and hatched into tanks of native stream water. Previously unreported behaviour of newly hatched tadpoles was observed. Observations were made through a series of tests for substrate preference (gravel, rocks or leaves), shelter or open water preference, and surfacing behaviour under different depths and turbulence levels. Tests found that tadpoles showed a preference for stream substrates over plastic tank floor, with the highest percentage of tadpoles found in gravel. Tadpoles preferred sheltered areas of the tank, and surfaced significantly less when water was turbulent, with depth having no significant effect on surfacing behaviour.

INTRODUCTION

The Tobago glass frog, *Hyalinobatrachium orientale tobagoense* is a subspecies endemic to the island of Tobago (Jowers et al., 2014). They reproduce by laying a clutch of gelatinous eggs on the underside of broad leaves overhanging streams, a mode of reproduction which is characteristic of all *Hyalinobatrachium* species (Hoffmann, 2010). The embryos develop on the leaf until hatching when they drop into the stream below where they develop further, eventually emerging as metamorphs. Nokhbatolfoghahai et al. (2015) and Downie et al. (2015) reported on embryonic development, hatching, and tadpole growth and morphology in *H. o. tobagoense*. Initially lacking pigmentation, the tadpoles develop pigment over much of the body, darker on the dorsal side, but lacking on the tail fins and ventral body. The tadpoles' eyes are dorsal and very small, and they have very long tails, 64% of total length. Growth, at least under well provided laboratory conditions, is very slow: after six weeks, tadpoles had tripled in mass, increased in length by a little under 40%, but were still at Gosner (1960) stage 25, with no sign of limb buds. Hoffmann (2010) also reported very slow growth and development in two glass frog species which took well over 200 days to reach metamorphosis.

By contrast, several species of pond-dwelling tadpoles in Trinidad can reach metamorphosis in less than 20 days (Downie, 2013). The elongated tadpoles of glass frogs are adapted to a fossorial lifestyle, presumably to escape the rigours of currents and predators in streams (Haddad & Prado, 2005; Hoffmann, 2010). They hide superficially in sediment and among rocks, gravel and fallen leaves, and escape with bursts of fast swimming when disturbed. Given their secretive nature, it is not surprising that the behaviour of these tadpoles is poorly known. The tadpoles grown by Downie et al. (2015) were never observed in the water column and were 'only retrieved after removal of all the rocks and gravel'. However, in preliminary experiments, recently hatched Tobago glass frog tadpoles were observed darting quickly to the water surface and back down again. In this paper, we report on glass frog tadpole surfacing behaviour in relation to water depth and turbulence. We also look at tadpole substrate preference when faced with a choice between three kinds of natural substrate and an empty plastic surface.

MATERIALS AND METHODS

All research and collections were conducted under a Research Permit granted by the Tobago House of Assembly, Department of Natural Resources and the Environment, to carry out research across north-east Tobago from June 09 2014 until August 31 2019, on *H. orientale* and other local amphibian species. Fifty six *H. o. tobagoense* egg clutches were collected from transects along five small forest streams in the north-east of Tobago over eight weeks from June to August 2016. The section of leaf on which the clutch lay was cut and stuck clutch side down in a petri dish, with damp cotton paper below it. Once brought back to the laboratory, clutches were stapled through the leaf clutch side down, to a cover on the lid of a clear plastic aquarium tank (29 cm × 18 cm × 16.5 cm) with a petri dish filled with native stream water sitting directly below to collect the tadpoles upon hatching. The tanks were kept in dark closed cupboards at room temperature (27-28 ˚C). Ten of the clutches were used in pilot studies, and observational tests carried out on the remaining forty six as explained below. The tests were carried out in a room at 27-28 ˚C with no artificial lighting, during daylight hours.

Once 10 tadpoles out of a clutch had hatched into the bottom of the petri dish, they were transferred to one of two clear plastic tanks (29 cm × 18 cm × 16.5 cm). These tanks contained native stream water, collected on the day of hatching, to a depth of 12 cm, except when the experiment required a different depth. The water was not aerated for any tests apart from the turbulence test. The tank was prepared for the test before the 10 tadpoles were transferred, and the tadpoles were left to acclimatise to these experimental conditions for 24 hours, with observations beginning at 08.00 h the next morning. All tadpoles and any remaining clutch material or undeveloped embryos were released back to the stream they were collected from after the observations.

For surfacing behaviour, the number of times the surface of the water in the tank was breached by any of the tadpoles was counted for a ten-minute period. After a five minute interval to minimise observer attention drift, this was repeated six times, so 60 minutes of tadpole behaviour was recorded per clutch over 90 minutes. For these observations, we used an empty tank substrate. These observations were made at two different water depths, 6 cm and 12 cm, and under two conditions: calm water at 12 cm and turbulent water at the same depth. Turbulence was created by aerating the water using pipes connected to two Resun air pumps AC-9602 producing an airflow rate of 120L/H. The tubes attached to the pumps were inserted to the tank and held underwater, 3 cm from the bottom at either end. The pumps created a steady flow of water within the tank, with air bubbles rising to the surface. The tests were carried out on six groups of 10 tadpoles, each from different clutches.

For substrate preference, the positions of the tadpoles were recorded at 10 minute intervals for one hour. Each test was repeated six times, each repeat on a different ten tadpoles from a new clutch. To test which substrate tadpoles preferred out of gravel, leaves and rocks from their native streams, half of the floor of the tank was covered with one of the different substrates, while the other half was left empty. At 10 minute intervals, the number of tadpoles in open water was recorded, and the number of tadpoles positioned in or on the substrate was deduced from that number. This method was necessary as the tadpoles are highly transparent and difficult to see against a substrate, and to disturb the substrate to find them would disturb the tadpoles and likely cause them to move to another part of the tank. For this reason also, it was not possible to give the tadpoles a four way choice of substrates at one time. Each test was carried out on six groups of 10 tadpoles, each from different clutches.

To test whether tadpoles chose to position themselves under shelter, half the bottom of the observational tank was covered using 6 cm high opaque half pipes. At 10 minute intervals over one hour, the number of tadpoles in the open was counted, allowing the number under shelter to be determined as the remainder. The test was carried out on six groups of 10 tadpoles, each from different clutches.

Data were analysed using R software, version 1.0.44 (2016-11-30) with RStudio. ANOVAs and Confidence Intervals were used to test for significance in tadpole behavioural tests. Each model was run simply with just the explanatory variable in question and compared using the LogLik function with more complex models including other explanatory variables to test which model fit the data best. For tests with random effects the lme4 package was used.

RESULTS

Substrate Type

The most preferred substrate was gravel (95% CI= 0.952 to 0.999; n=6), then rocks (95% CI= 0.006 to 0.6264; n=6) and then leaves (95% CI= 0.0566 to 0.9601; n=6). When gravel, leaves and rocks are grouped as one explanatory variable, tadpoles were found significantly more on one of these substrates than on the empty tank floor (95% CI= 0.9534 to 0.9986). The overall percentage of tadpoles found in gravel was 97.8%, in rocks was 96.7% and in leaves was 86.9%.

Shelter Preference

Tadpoles were found significantly more under shelter than in open water (95% CI= 0.5742 to 0.6739; n=6). The mean percentage of tadpoles found under shelter was 62.5%. The majority of the sheltered tadpoles were located within the narrow gap between the two shelter components. This was recorded as an observation at the time of the experiment, but no precise figures were recorded.

Surfacing Behaviour: Turbulence Tests

A "surface" was recorded when a tadpole swam from the bottom of the tank to the surface of the water. The tadpoles swam fast, taking 1-2 seconds to get from bottom to surface. The majority of tadpoles would immediately dive down after surfacing, but it was observed on occasion that some would "bob" along the surface before diving back down.

The frequency of tadpole surfacing events decreased significantly when the pumps were on in the tank (mean =1.36 +/-1.51SD number of surfacings per trial with pump on; mean= 3.33 +/-1.77 SD number or surfacings per trial with pump off; $F_{1,51}$= 12.7294, p= 0.0007, R^2=0.46735; n=6).

Surfacing Behaviour: Depth Tests

Depth was found not to have a significant effect on tadpole surfacing frequency ($F_{1,55}$= 0.3901, p= 0.5345, R^2= 0.2319; n=6). The mean number of surfaces per group of 10 tadpoles, per 10 minute observation period, was 2.86 +/- 2.2SD at a depth of 6 cm, and 3.53 +/- 2.3SD at 12 cm. (Fig. 1)

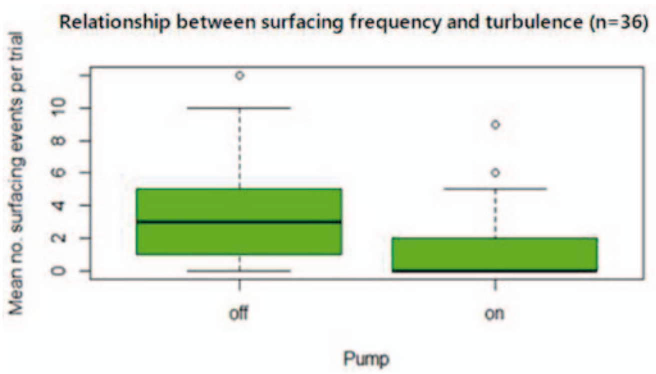

Figure 1. The differences in tadpole surfacing frequency with pump on versus pump off. 'Trial' here means 10 minute observation period. Clear circles represent outlying data points. Upper and lower horizontal lines represent minimum and maximum values. The median value is represented by the bold black line, within the green box which shows the interquartile range.

DISCUSSION

Because of the small transparent nature of *H. orientale* tadpoles, we used binary choice tests for substrate preference, establishing a strong preference for rocks, gravel or leaves over an empty plastic substrate, with some evidence of a preference for rocks or gravel over leaves. It would be inappropriate to assess the significance in differences between the three natural substrates, given that the choices offered were binary. Hoffman (2010) reported that most *Hyalinobatrachium* species hide between rocks, stones and coarse river gravel, according with our results. As is commonly the case (Altig et al., 2007), the *H. orientale* tadpole diet is unknown, but their preferred habitat along with their rasping mouthparts (Downie et al., 2015a) suggests biofilm grazing.

It was interesting that, although there was a preference for shelter over lack of shelter, this was not as strong as in the substrate tests. This may have been because of the empty plastic substrate available for both choices. It was noticeable that most of the sheltered tadpoles were not located simply under the shelter, but in the narrow gap between two shelter components, suggesting that the strong preference is for a narrow space. This also suggests that the shelter provided for the tests may not have been perceived as shelter by the tadpoles. Hoffman (2010) describes centrolenid tadpoles as using their flexible bodies and tails to sequester themselves into narrow cracks between stones and rocks, similar to the narrow crevice between the two half-pipes.

Some species of tadpoles swim to the water surface to fill their buccal cavity with air, then quickly swim back down again (Feder & Catherine, 1984). Tadpoles are sensitive to environmental conditions, and alter their behaviour in response to stressors; for example, between a bare, lit environment, compared to shade or partial shade (Michaels & Preziosi, 2015). This response can be manifested by decreasing activity or hiding (Semlitsch & Reyer, 1992). It was initially a surprise that *H. orientale* tadpoles surfaced at all, given their secretive nature and habitat, running water, where oxygenation should be high. However, given that surfacing was observed we expected that *H. orientale* tadpoles would surface less in deeper or turbulent water, as energy expenditure and risks of predation or being swept downstream would be higher. Surfacing frequency, however, was not different at depths of 6 cm compared to depths of 12 cm. Perhaps a bigger depth variation might show a difference, but in the shallow streams where these tadpoles live, the depth variation we used is relevant to real life conditions.

We expected that tadpoles would surface less when the pump was on and creating turbulence in the tank, as it would simulate a faster flowing stream which could sweep tadpoles downstream. This result was observed during the behavioural tests. In streams, *H. orientale* tadpoles may use a form of flow sensing, similar to *Xenopus laevis* tadpoles which use their lateral line organs to sense their environment (Simmons et al., 2014), and alter their behaviour, in this case surfacing, accordingly. The heterogenous environments which *H. orientale* inhabit are heavily affected by abiotic factors, such as increased turbulence, flow and depth of stream water from rainfall, and these may impose selective pressures on *H. orientale* tadpole behaviours which are not yet well understood. These pressures may result in the behavioural flexibility seen in the surfacing frequency tests, and these tests provide a baseline of previously undescribed behaviours for tadpoles of this species. It is possible that the difference in surfacing between calm and turbulent water reflected differences in oxygenation. We consider it unlikely that the fresh stream water used in our experiments became sufficiently de-oxygenated to make a difference in the calm water test. We did not have access to an accurate oxygen meter to test this possibility.

Tadpole behaviour is not well studied across all species, and even a basic knowledge of diets is still lacking. Our observations were restricted to newly hatched tadpoles; it would be of value to investigate how behaviour changes as individuals grow larger as they approach metamorphosis. Considerable work is needed on enigmatic groups like Centrolenidae (McDiarmid & Altig, 1999), and there are very few data available on the natural history of *H. orientale* (Lehtinen & Georgiadis, 2012). Understanding the trophic status of tadpoles and their ecological roles is especially important in light of the current and rapid global amphibian decline. Knowledge of their natural histories and trophic interactions are increasingly required for effective conservation and predictions of the consequences of their possible losses, and to determine their functional roles within the ecological communities they inhabit (Altig et al., 2007). The behaviours and preferences described in this report are previously undocumented for the tadpoles *H. o. tobagoense* and, to the best of our knowledge, for other *Hyalinobatrachium* species. It is hoped that these insights may shed some light on the stream microhabitats which are crucial to glass frog development.

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