

Captive husbandry and breeding of file-eared tree frogs, *Polypedates otitophus* (Boulenger, 1893) (Amphibia: Anura: Rhacophoridae)

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ABSTRACT - Six *Polypedates otitophus* were reared from small juveniles to adult breeding size over a period of 18 months. An account of captive husbandry and breeding is provided. Clutch size ranged from 44 – 119 eggs. Eggs hatched after ten days and tadpoles attained total lengths of 85 mm. Metamorphosis took 74 – 84 days at 22 – 26 °C.

INTRODUCTION

The file-eared tree frog (*Polypedates otitophus*) is a large nocturnal rhacophorid frog from in Borneo and Java. (Inger et al., 2004; Inger & Steubing, 2005; Riyanto et al., 2009). Matsui et al., (2014) recently split the species on morphological and genetic grounds, the populations in Sumatra are now assigned to *P. pseudotitophus*. *P. otitophus* is an occupant of primary and disturbed forest and tea plantations from 0 - 1, 500 meters above sea level (Malkmus et al., 2002; Inger & Steubing, 2005). *P. otitophus* feed on invertebrates and have been observed consuming small sympatric *Rhacophorus dulitensis* (Sheridan et al., 2012). *P. otitophus* is a foam nesting species and breeds along or above streams and pools which are usually turbid (Tapley B. pers. obs). Eggs are laid in foam nests which are suspended 30 cm above the surface of the water on low plants (Malkmus et al., 2002). When eggs hatch the tadpoles drop into the water below.

Lifespan and age at sexual maturity in the wild have not been reported. The maximum lifespan in captivity is also unknown. Iskandar (2004) states that *P. otitophus* does not do well in captivity although details of captive management were not reported. Despite being Least Concern this species is poorly known in the wild and in captivity (Inger et al., 2004).

METHODS

Six captive-bred juveniles of this species were obtained in September 2012 from a private breeder in Hungary. Specimens measured 50 mm SVL at the time of acquisition. Two individuals exhibited malformation of the spine and the breeder stated that the frogs had not been exposed to UV-B radiation. The cause of the malformation could have been developmental or due to inappropriate nutrition and/ or lighting.

The group was initially housed in a 40 x 40 x 40 cm glass vivarium with a mesh panel in the lid. As this frog is an arboreal rainforest species it is likely to be exposed to UV-B radiation in day time retreat sites. UV-B radiation is important for the synthesis of vitamin D₃ which plays an important role in calcium metabolism, muscle development, organ formation, muscle contraction and immune and nervous system function (Whitaker & Wright, 2001). Captive *P. leucomystax* develop metabolic bone disease when UV-B radiation is not provided, even when prey items were dusted with vitamin D₃ containing supplements (Tapley B, pers. obs.). UV-B provision was therefore considered important for the captive maintenance of *P. otitophus*. This was provided by mounting a UV-B emitting lamp (1150 mm T5-HO UV-B) fluorescent tube (D₃+ 12%UV-B Reptile Lamp, Arcadia Products plc, Redhill, UK) above the mesh of the vivarium top. UV Index (UVI) readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter, UVI gradients were measured through the mesh lid and ranged from a UVI of 0 – 4.0 at the level of the frogs' dorsal surfaces. The floor of the vivarium had a built-in aquatic area which measured 40 x 12 cm and was filled with aged tap water to a depth of 3 cm. Compacted organic peat free compost was used as a substrate. A single bird's-nest fern (*Asplenium antiquum*) provided a resting site for the juvenile frogs. Branches were angled from the water into the back top corners of the vivarium and it was on these branches that the highest UVI reading (UVI 4) was measured.

The frogs rapidly outgrew their enclosure. When the largest individual measured 65 mm SVL, all specimens were transferred to a custom-made 100 x 75 x 35 cm breeding vivarium (Custom aquaria, Rushden, UK). The tank was designed to recreate the breeding sites of this frog; a pool with overhanging plants and branches. The base of the vivarium was filled with aged tap water to a depth of 20 cm. *Ficus binnendijkii* and *Dracaena compacta* were planted into two planting compartments. Branches and

fronds of the lady palm (*Rhapis excelsa*) were added for additional resting sites. A mesh panel in the roof of the vivarium allowed for the same lighting array described above. UVI readings were taken on a monthly basis with a Solarmeter 6.5 UV Index Meter and were measured through the mesh lids and ranged from UVI of 0 – 6.0 at the level of the frogs' dorsum. Photo period was 12:12 all year round. In the non-breeding season, full water changes were carried out every week with aged tap water. The water was heated to 27°C with a 300 W aquarium heater (All Pond Solutions Ltd, West Drayton, Middlesex), this was to maintain high ambient humidity and to keep temperatures stable in the enclosure as there was little temperature control in the room in which these frogs were housed. Ambient temperatures within the tank ranged from 23°C and 26°C (night/day summer) to 20°C and 25°C (night/day winter), and temperatures of 28°C were recorded on the branches directly below the UV-B emitting lamp, the highest temperature in the enclosures therefore overlapped with the greatest UV-B radiation which replicates what we would expect to occur in nature. The overlap of the highest temperature and highest UV-B level is important as the formation of D₃ is dependent on the thermal isomerisation of its precursors (Webb, 2005; Tapley et al., 2015).

The enclosure was lightly misted with aged tap water at approximately 21:00 hrs on a daily basis. The enclosure and all furnishings were thoroughly scrubbed with a brush and water on a weekly basis. No chemical cleaners or disinfectants were used.

Juvenile specimens were fed every three to four days on live invertebrates, predominantly crickets (*Gryllus assimilis* and *G. bimaculatus*). Feeder insects were released into the enclosure in the evening corresponding with the frogs' peak activity time. This was considered important as the food insects were consumed when they were still coated in the dietary supplement. Once the frogs attained a SVL of 65 mm they were occasionally offered 4th instar locusts (*Schistocerca gregaria*) and cockroaches (*Dubia blaptica*). Prior to being offered to the frogs, all feeder insects were placed in a polythene bag and dusted with a high-calcium multivitamin and mineral supplement containing vitamin D3 (Nutrobal, Vetark Ltd., Winchester, UK). Approximately 8 prey items were offered to each frog at each feeding event.

RESULTS

The only male began vocalising in mid-February 2014. Female specimens were frequently observed toe tapping in response to the vocalisations of the male. This has been documented in other *Polypedates* species; *P. leucomystax* females toe tap to attract vocalising males, in response to the toe tapping male frogs move closer to, and eventually locate the females (Narins, 1995). Our captive *P. ottilophus* spawned from April until November. As the females were not individually identifiable and because we did not observe all egg laying events, inter-clutch intervals

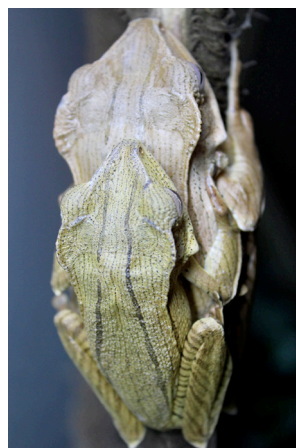


Figure 1. Amplexant *P. ottilophus*.



Figure 2. Foam nest deposited on *F. binnendijkii*.

and the number of clutches per female was not recorded. Amplexus is axillary (Fig. 1). Fourteen fertile foam nests were produced by the five females from April 10th - November 4th 2014. Egg laying was observed to take place five times in the early morning at approximately 06:00 hrs. Three of the nests were deposited on *F. binnendijkii* leaves (Fig. 2), and 11 clutches were deposited on the glass sides of the vivarium. On 19th May 2014 the process of nest construction was observed in its entirety, the process took approximately 45 minutes. Once the foam nest had been constructed the female created a chamber in the centre of the nest using circular motions of her hind feet, eggs were then deposited in the cavity in the centre of the nest.

Eggs were left in-situ because we believed that oviposition sites selected by the frogs were likely to be optimal for egg development. Three nests were dissected within 24 hours of laying and contained 42 - 119 eggs. Foam nests were sprayed on a daily basis with aged tap water. A receptacle containing shop bought bottled mineral water (2 cm deep) was secured directly below the foam nest to catch the tadpoles as they hatched. Larvae emerged from the foam nest approximately 10 days after laying and most still had large yolk sacs.

Larvae were kept in the receptacle for 48 - 72 hours until they were free swimming and then transferred to a 30 x 20 x 20 cm (12 litre) tank, without substrate, for rearing. Shop bought bottled mineral water was used for rearing as the local tap water was extremely hard and not deemed appropriate for the species as in nature they occur in microclimates fed by rain water which is normally soft (Poole & Grow, 2008). Oak leaves (*Quercus robur*) were added to acidify the water. Water parameters were as follows: pH: 7.5, KH: 80-120 mg/L, GH: 120 mg/L. Air stream sponge filters were used for mechanical and biological filtration. Twenty percent water changes were carried out on a weekly basis. Filter medium was cleaned and rinsed in the water removed during the water change at each partial water change. Water was heated with a 50 W aquarium heater (All Pond Solutions Ltd, west Drayton, Middlesex). Water temperatures during the larval period ranged from 22°C – 26°C. Larvae were fed ad libitum every day on commercial fish foods, Tetra Pro Colour and

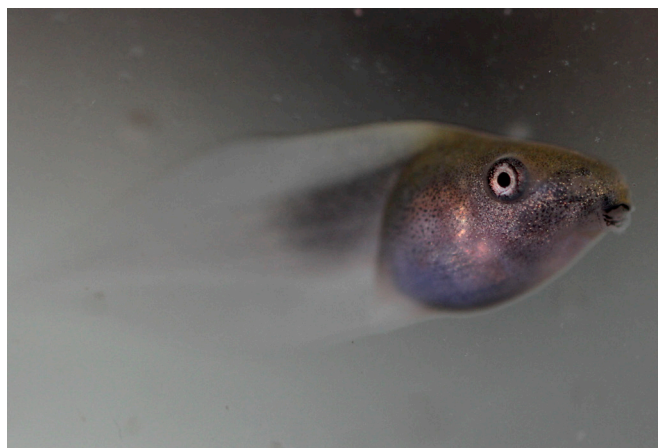


Figure 3. *P. ottilophus* larvae prior to the development of the characteristic longitudinal stripes.



Figure 4. Metamorph *P. ottilophus* exhibiting characteristic longitudinal stripes.

Tetra Pro Algae (Tetra Werke Melle, Germany) and Hikari algae wafers (Kyorin Food Industries, Ltd. Japan).

When the larvae exceeded 60 mm (Fig. 3) they were deemed too large for the rearing tank and they were transferred to the aquatic area in the adult frogs' enclosure (40 litres), water parameters were the same as above. Two weeks prior to metamorphosis, larvae developed the characteristic longitudinal dark stripes and at this point attained a total length of 85 mm. The first larva metamorphosed 74 days after hatching (Fig. 4), all of the clutch metamorphosed within 10 days.

Metamorphs were housed in the same set up used to house the 50 mm juveniles that were originally acquired. Metamorphs were offered *Drosophila* and 1st instar crickets (*G. assimilis* and *G. bimaculatus*). Prior to offering to the frogs, the feeder insects were placed in a polythene bag and dusted with Nutrobal®. Specimens started feeding 10 days after the tail had fully resorbed. Metamorphs were fed daily until six weeks of age after which, the feed interval was gradually increased to once every three days.

DISCUSSION

Clutch size was smaller in *P. ottilophus* than documented in most other *Polypedates* species. Mean clutch size of *P. leucomystax* in Thailand was 476.94 and in Singapore 261.49 (Sheridan, 2008). *P. leucomystax* is likely to be a species complex and variation in the clutch size of *P. leucomystax* could be attributed to undocumented diversity within the species. Clutch size in *P. ottilophus* was also smaller than recorded in *P. maculatus*, which ranged from 275 – 719 (Monhanty & Dutta, 1988) and *P. braueri* where clutch size ranged from 400 – 500 (Yang, 1998). *P. ottilophus* egg diameter was not measured and it is not possible to state whether or not *P. ottilophus* produce fewer but larger eggs than other *Polypedates* species.

Malkmus et al., (2002) report that in the wild, *P. ottilophus* larvae attain a total length of 80 mm. Captive reared larvae exceeded this and attained a total length of 85mm, considerably longer than published reports. We believe 85mm to be the greatest total length reported for any *Polypedates* larvae. Larvae of *P. colletti* reach 32.8

mm total length (Hass & Das, 2008), *P. leucomystax*; 50 mm (Malkmus et al., 2002), *P. macrotis*; 60 mm (Malkmus et al., 2002; Inger and Steubing, 2005) *P. maculatus*; 50 mm (Daniels, 2005) and *P. teraiensis*; 47mm (Tamuly & Dey, 2014). Larvae of some *Polypedates*, however, remain undescribed and may grow larger.

Oviposition in *P. leucomystax* was not dependent on recent rainfall (Sheridan, 2008) and, in captivity, *P. ottilophus* were bred without the use of a rain chamber. Reproductive activity did not appear to be temperature dependent as they continued to produce fertile eggs when the temperature of the enclosures had dropped from 23°C and 26°C (night/day) to 20°C and 25°C (night/day) in November 2014.

To our knowledge this is the first documented breeding of this species in the UK. Wild *P. ottilophus* are still collected from the wild for the international pet trade (Tapley B, pers. obs.). The global trade in amphibians and the lack of associated biosecurity may facilitate the spread of amphibian pathogens (Garner et al., 2009; Martel et al., 2014). The methods outlined here could be adopted in order to establish this and other *Polypedates* species in captivity which may reduce the number of *Polypedates* of wild caught origin being traded.

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