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Preliminary observations on population structure, mating behaviour, and the fig-feeding tadpoles of the Hourglass Toad, *Leptophryne borbonica* (Tschudi, 1838), from Malang, East Java Province, Indonesia

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Abstract. Studies on the status and natural history of Javan frog population are scarce, making it difficult to assess population stability. During 2020–21, we monitored breeding sites of the Hourglass Toad, *Leptophryne borbonica* Tschudi, 1838, in Malang, East Java. Our aim was to assess the structure of this population, the microclimate under which it operates, and any discernible life history traits. We found that population size fluctuated monthly during the rainy season, with adult males at their highest number in the middle of rainy season (March). We observed an axillary amplecting pair and tadpoles feeding on figs. Our monitoring provides insights into the logistics of in-situ population assessments of Javan frogs, and our approach is particularly relevant for conservation programs threatened congener.

Keywords. Anura, exotrophic tadpole, natural history, seasonal breeder, sex-bias

Introduction

Herpetological studies on Java, Indonesia, have been conducted since the colonial period in the early nineteenth century (e.g., Horst, 1883; Dunn, 1928; Schijfsma, 1932). However, the majority of studies on Java's frogs has focused on species discovery, particularly on cryptic species that are frequently observed in this region, including the two recent examples of *Leptophryne javanica* Hamidy et al., 2018 and *Chirixalus pantaiselatan* Munir et al., 2021. In contrast, other fields of study, such as ecology, behaviour, and reproductive strategy, have advanced much more slowly and received comparatively little attention (Iskandar, 2020). This includes population surveillance and long-term census data in our study area (Kurniawan et al., 2021; Kusrini et al., 2021).

As the central administrative hub of Indonesia with some of the world's most densely populated urban areas, Java faces serious and inescapable environmental threats, such as habitat loss (Kurniawan et al., 2021). Additionally, there are too few studies that focus on long-term monitoring of frog species (Kusrini, 2005), including population monitoring of the *Fejervarya limnocharis-iskandari* complex, *F. cancrivora* (Gravenhorst, 1829), and *Limnonectes macrodon* (Duméril & Bibron, 1841), as well as members of the genus *Leptophryne* from Mt. Gede Pangrango, Mt. Ciremai, and Mt. Slamet in West Java and Central Java Provinces (Kusrini et al., 2007; Hamidy et al., 2018). As a result, our ability to determine the difference between population declines and natural fluctuations in Java's frog diversity is severely limited. Given the paucity of information, population studies and observation of life history traits are required to mitigate the imminent threat of anuran extinctions in Java as a result of the rapid growth of the island's human population.

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East Java Province is one of the most overlooked regions of Java, with scant survey effort on herpetofaunal diversity in comparison to neighbouring West Java and Central Java Provinces, and yet East Java is considered to have the highest rate of deforestation (Kurniawan et al., 2021; Kusrini et al., 2021). The earliest explorations of East Java's herpetofauna occurred in the early 1990s (Hodges, 1993) and more recently, between 2018 and 2020, in Raden Soerjo Forest Park and Bromo Tengger Semeru National Park (BTSNP), which revealed a rich diversity of species (Indrawati et al., 2018; Septiadi et al., 2018; Arroyyan et al., 2020; Kurniawan et al., 2021). Due to the fact that many frogs in Java are primarily restricted to specific microhabitats, lack reported life histories, and are prone to local extinction, there is an urgent need for a long-term census to ensure populations sustainability.

Leptophryne is a genus widely distributed from Peninsular Thailand through the Malay Peninsula and into the Greater Sunda Islands (Iskandar, 1998; Poyarkov et al., 2021; Frost, 2023). The genus comprises three species, (1) *Leptophryne borbonica* (Tschudi, 1838), widely distributed across rainforests of Peninsular Thailand through Malaysia into Sumatra, Borneo, and Java; (2) *L. cruentata* (Tschudi, 1838), known only from Mt. Pangrango, Mt. Gede, and Curug Luhur of West Java; and (3) *L. javanica* Hamidy et al., 2018, known from Mt. Slamet in Central Java and from Mt. Ciremai and Mt. Sawal Wildlife Reserve in West Java (Cahyadi and Noerwana, 2021). Members of this genus generally inhabit wet areas or clear, slow-moving waters (Iskandar, 1998) and the leaf litter of seepage areas in secluded forests (Inger 1966), which may be sensitive to environmental stressors (Ardiansyah et al., 2014).

Numerous studies have been conducted on members of the genus *Leptophryne* in Sumatra and Java, including on the distribution, morphology, and ecology of *L. borbonica* and *L. cruentata* on Mt. Gede Pangrango in West Java (Kusrini et al., 2007), the abundance of *L. borbonica* on the same mountain (Ardiansyah et al., 2014), the extended distribution, microhabitat, and morphology of *L. borbonica* in Malang Regency in East Java (Erfanda et al., 2019), and the microhabitat of *L. borbonica* in Sumatra (Nugraha et al., 2021). However, there is still no information on the population status and natural history of *L. borbonica*. This study is the first to address the population structure, microclimate, and life history traits of *L. borbonica* in Malang Regency of East Java.

Materials and Methods

Study Area. Erfanda et al. (2019: 87) suggested that the southwestern side of Bromo Tengger Semeru National Park (BTSNP) was the only known breeding site of *L. borbonica* in East Java (ca. 7.59°S, 112.48°E; coordinates are approximate to protect the specific locality). Thus, we select this area as our study area, considering that the area is within the buffer zones of the national park, which is prone to human disturbances, agroforestry, and nature tourism activities. The breeding site was described as a small, shallow, and slow stream, which discharges into a wide, deep river followed by several waterfalls. The canopy is dense so that sunlight cannot fully penetrate to the breeding site (see Erfanda et al., 2019: p.86).

Identification. We validates species identity by using the morphological characters previously described for *L. borbonica* (Inger, 1966; Iskandar, 1998; Kusrini et al., 2007; Erfanda et al., 2019). For sex-determination and size class assignment, we categorized adult males by the presence of median subgular vocal sacs, nuptial pads, and snout–vent length (SVL) ranging from 21.0–28.0 mm; adult females by larger SVL, ranging from 28.0–32.0 mm (see Fig. 1); and juveniles for any individuals with SVL < 20.0 mm. For tadpole identification, we used the morphological characters defined by Iskandar (1998) and Erfanda et al. (2019). We adopted the staging tables of Gosner (1960) to define the tadpoles' stages.

Population monitoring and observation. We sampled the study site monthly between December 2020 and June 2021 to reflect seasonal factors (e.g., rainy season), with the exception of the period April–May 2021, when a landslide near the site prevented access. A 45-m-long transect was established for population monitoring along the small stream, with a river depth of 10–30 cm and width of 1.5 m (elevation 850–870 m). During each sampling, three to four surveyors were equipped with headlamps, boots, and other standard safety equipment. To minimize disturbance to the population, we traversed the designated transects from downstream to upstream by standing on rocks or solid substrates and avoiding direct contact with the streambed. The species' presence was indicated by the calls of males, and we counted all individuals seen along the transect until no individuals remained (Table 1). Each frog was captured by hand and placed in a separate plastic bag. After observations and measurements were completed (SVL and sex), frogs were immediately released into the same location. Prior to conducting the surveys, our equipment was disinfected to minimize the spread of diseases, such as chytridiomycosis, which has been reported from West

Figure 1. Size comparison between adult male (right) and adult female (left) Hourglass Toads, *Leptophryne borbonica*, from Malang, East Java, Indonesia. Note that the body and SVL of the female are bigger than of adult male (right). Photo by A.M.S.P. Soeprijadi.

Table 1. Number of Hourglass Toads, *Leptophryne borbonica*, observed during each survey (tadpoles – T, juveniles – J, males –M, females – F), including a list of syntopic species. The asterisk (*) indicates that the observed tadpoles possibly belonged to a mixed set of species.

Date	Time	J	M	F	T	Syntopic Species
2 December 2020	17:00–19:00 h	1	9	0	> 100*	*Chalcorana chalconota, Leptobrachium hassel Limnonectes microdiscus, Megophrys montana, Microhyla* sp., *Philautus aurifasciatus, Wijayarana masonii*
26 January 2021	17:00–18:00 h	0	7	1	0	*Chalcorana chalconota, Megophrys montana, Limnonectes microdiscus*
9 February 2021	12:00–14:00 h	0	8	0	0	None
22 March 2021	14:00–15:30 h	0	20	0	0	*Leptobrachium hasseltii*
April–May 2021	no observations					
9 June 2021	10:30–11:30 h	0	10	1	> 100	*Limnonectes microdiscus*

Java (Kusrini et al., 2008). During the observations, we record humidity, dew point, and air temperature using a Benetech GM1365 data logger placed in the transect's middle point (1 m above the ground).

We carefully observed and photographed an amplecting pair, the presence of eggs or tadpoles, and other syntopic species (in adult form) present at breeding sites to study life history traits. We noted the riparian vegetation (e.g., bamboo, fig tree, shrubs) as potential shelters of food sources for tadpoles and identified them using the available literature (Berg et al., 2005).

Data analysis. Using SVL measurements, we classified toads into size classes and by sex. Temperature, humidity, and dew point recorded in the data logger were downloaded using the included software. The abundance of individuals was interpreted against each of these microclimatic variables (i.e., humidity, temperature, and dew point) throughout observation periods using Microsoft Excel 365.

Results

Population monitoring and environmental fluctuations. Our observation showed that the abundance of *L. borbonica* fluctuated across all life stages and seasons (Table 1; Fig. 2). From December 2020–June 2021, the number of adult males was highest compared to adult females and juveniles, of which we found only one individual each. The total number of adults was quite stable from December 2020–February 2021 (7–9 individuals/month) but increased to 20 individuals during March 2021. This difference was seen in all stages, with the highest adult counts occurring in March 2021 (rainy season) and with an estimated peak during the following, unobserved months (April–May 2021). Individual occurrences began to decline in June 2021 (late rainy season).

We estimated that more than 100 Hourglass Toad tadpoles were present in December 2020 and June 2021 (Table 1). However, we could not confirm whether all the tadpoles seen in December 2020 were Hourglass Toads, since there is likely a mix of tadpoles from different species present at the site. Tadpoles observed in June 2021 (Fig. 3) matched the description of Hourglass Toad tadpoles perfectly, and egg masses were guarded by adult toads. The environmental variables (i.e., temperature, relative humidity, dew point) varied across all sampling dates and times but showing a definite increase approaching March 2021 (Fig. 2).

Observation of amplexus. In June 2021, we observed an amplecting pair of Hourglass Toads during the daytime at 10:39 h (Fig. 4). The pair was observed on level, moist, mossy substrate, not far from the small stream and shallow water of the breeding site. We observed axillary amplexus, with the male's first and second finger resting above the female's axillary region, his head resting on the female's interorbital region, and his hind limb resting on the female's femur. Amplexus was observed for over an hour and remained intact when we left the observation site. During amplexus the toads remained still and appeared to avoid contact with other individuals.

Observation on tadpole feeding behaviour. On 9 June 2021, we estimated that > 100 Hourglass Toad tadpoles were present in a small, shallow, clear, and slow-moving stream with a depth of 20 cm. The majority of the tadpoles were around Gosner stages 26–30. The stream bed at this site was composed of sand, gravel, and leaf litter. The tadpoles were gathered around a fallen fruit of *Ficus lepicarpa* with diameter of ca. 18 mm that had begun to rot and developed a mushy texture (Fig. 4). Fruits of these fig trees fall into the river regularly and the tadpoles access the submerged fruit. Tadpoles appeared to be eating from both the inside and outside of the hollow fruit by scraping the fruit surface at one position, then wiggling their tails to move to an adjacent surface. This observation allows us to hypothesize that Hourglass Toad tadpoles are exotrophic tadpoles.

Discussion

The only long-term monitoring study for the genus *Leptophryne* to date was conducted by Kusrini et al. (2007). They compared *L. cruentata* specimens collected in 1964 to their own observations on Mt. Gede Pangrango in West Java in 2006 and concluded that the population was declining. In light of these findings, Ardiansyah et al. (2014) examined the correlation between abundance and environmental factors affecting Hourglass Toads on that mountain and discovered only weak correlations, implying that other factors may be involved. As a result, it is critical to establish data on frog abundance in order to better understand the needs of *Leptophryne* populations.

Our observations indicated that successful breeding of Hourglass Toads likely occurs in March–June 2021, evidenced by a high number of tadpoles at the end of rainy season (June 2021). The breeding season likely began in the previous month, for which we were not able to make detailed observations due to the landslide. Given the environmental data (see below), we surmise that seasonal factors played a role in the fluctuations of Hourglass Toad abundance. According to Duellman and Trueb (1994), amphibians have a wide variety of reproductive strategies (e.g., seasonal breeder, continuous breeder, opportunistic breeder), and most tropical frogs reproduce seasonally in response to rainfall timing (Othman et al., 2011). According to Kusrini et al. (2007), *L. cruentata* employs seasonal breeder strategies by releasing all eggs in a single clutch, and this is also how Hourglass Toads breed (Iskandar et al., 1998).

Our abundance and microclimate data support the hypothesis that Hourglass Toads are seasonal breeders with a mating season that in 2021 began in February–

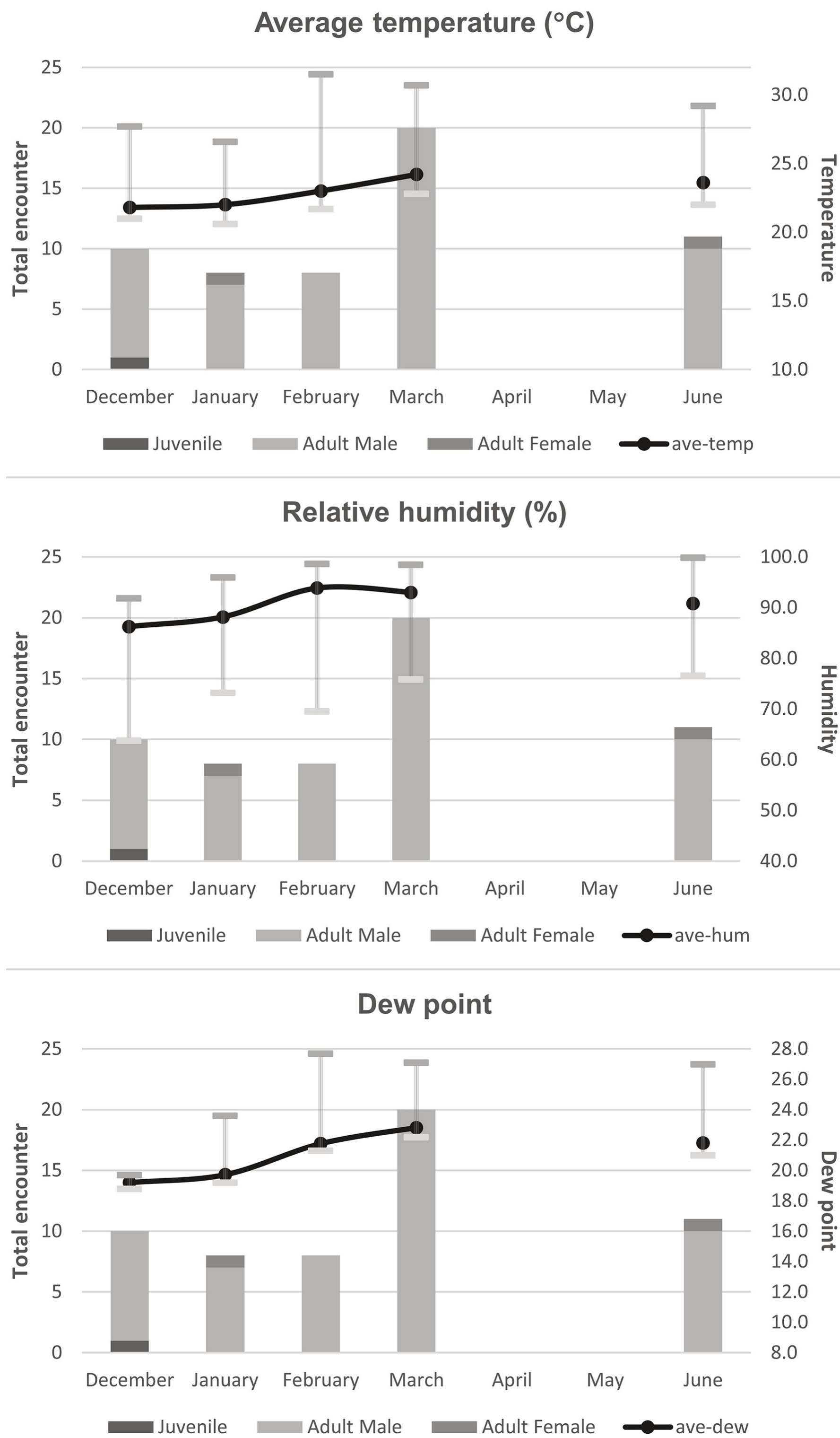

Figure 2. Three microclimatic variables (temperature, humidity, and dew point) plotted against the abundance of Hourglass Toads, *Leptophryne borbonica*, in Malang Regency, East Java, Indonesia, throughout the sampling period (December 2020–June 2021). The absence of data between April and May 2021 was due to survey difficulties caused by a landslide near the site.

March (mid- to late rainy season). To confirm this, additional data on reproductive data (clutch size, gonad size) are required. We observed no tadpoles in our January–March surveys (early rainy season). This absence could be a result of low rainfall (Smith, 1983), interspecific competition (Strauß et al., 2016), predation and parasitism (Alford, 1999), or a lack of food sources (Alford, 1999; Kloh et al., 2018). Throughout the early- to mid-rainy season, numerous food sources (e.g., fig fruit) were washed away by heavy rain, resulting in flooding. As a result, hundreds of Hourglass Toad tadpoles were observed during the late rainy season (March 2021), when rainfall was reduced. Annual data are required to confirm whether the population of Hourglass Toads in Malang is declining.

The most active period for Hourglass Toads, which may be nocturnal, diurnal, or ephemeral (Duellman and Trueb, 1994), is unknown and this may skew our results on population abundance. However, our observations using a variety of sampling times (Table 1) indicate that the frogs can be observed at any time of day but are most easily encountered at night due to their louder calling (which is quieter during the day) and optimal time for preying. We assumed that if the survey were conducted at night, there would be a greater abundance during the rainy season (February, March, and June 2021), demonstrating clear seasonal effects. Our observations indicated that the presence of heavy rain prior to the survey had a detrimental effect on frog encounters (absence of individuals). The preceding rain

Figure 3. Several Hourglass Toad, *Leptophryne borbonica*, tadpoles in Malang, East Java, Indonesia, seen scraping the surface of the fruit of a fig tree (*Ficus lepicarpa*). Photo by S.R. Devi.

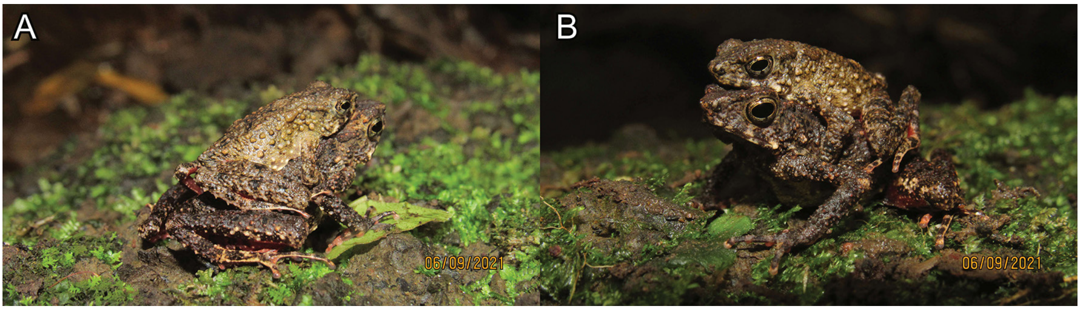

Figure 4. Amplexus between adult male (top) and adult female (bottom) Hourglass Toads, *Leptophryne borbonica*, from Malang Regency, East Java, Indonesia, in rear (A) and frontal views (B). Note that the male's first and second finger were placed above the female's axilla, and the head of the male rested on the female's interorbital region. Photos by S.R. Devi.

may have compelled frogs in breeding sites to seek a safer haven away from the fast-moving water.

Male-biased abundance was observed in Hourglass Toad populations (Table 1; Fig. 2), which is considered to be common among anurans (Madsen and Loman, 2010) due to the differing sex behavior of males and females. Males are typically the first to arrive at breeding sites, make advertisement calls, are capable of breeding multiple times, and are the last to depart (Kuhn, 1994). As a result, males are relatively easy to observe and capture. Females, on the other hand, behave differently: they are silent, cryptic, and spend only enough time mating and laying their eggs once (Kruse and Mounce, 1982). Hourglass Toad abundance is also male-biased in Sumatra (Nugraha et al., 2021) and West Java (Kusrini et al., 2007).

Our observation of the amplexus in Hourglass Toads was consistent with a previous report from Sumatra (Nugraha et al., 2021), with the female attached to the rock substrate via the axillary amplexus. However, information about the amplexus type of the Bleeding-toad and Sumatran tree toad remained sparse. Our observation of the Hourglass Toad feeding behaviour demonstrates the critical role of surrounding vegetation, particularly the fig-fruit of *Ficus lepicarpa*, as a food source for tadpoles. We believe that with additional sampling in East Java, local distributions of this species will be discovered, revealing additional essential food sources for the tadpoles. Due to the paucity of information on the tadpole in nature and other aspects of biodiversity (Altig et al., 2007; Iskandar, 2020), future conservation efforts may be slashed.

Several threats to the *L. borbonica* population have been reported, including massive anthropic landscape changes, volcanic activities, and unsustainable ecotourism (Erfanda et al., 2019), but the species is still categorized as Least Concern under IUCN Red List criteria due to its wide distribution (IUCN SSC Amphibian Specialist Group, 2021). Another potential threat is infection by *Batrachochytrium dendrobatidis* (Bd), a fungus that has been a significant factor in a global decline amphibians, which has previously been reported to be present in West Java, where it is infecting several frog species (Kusrini, 2008). However, the extent of Bd in East Java remains unknown and requires further studies.

Our monitoring of Hourglass Toads in Malang Regency, East Java demonstrates the effect of seasonal factors and identifies novel life history traits. Additional monitoring efforts on this and other poorly known frog species are recommended in order to elucidate further aspects of their biology. Even though our findings are preliminary, they provide an important baseline for conservation programs that can be applied to this species and threatened congeners, such as *L. cruentata* and *L. javanica*.

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