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Cover: Golden-headed Lion Tamarin *Leontopithecus chrysomelas*. Watercolor and acrylics by P. Kritika.



Habitat associations and feeding ecology of adult Tamdil Leaf-litter Frog *Leptobrachella tamdil* (Amphibia: Megophryidae) from the type locality – the Tamdil wetland, Mizoram, India

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Abstract: We conducted a study on the habitat use, food, and feeding ecology of the lesser known Tamdil Leaf-litter Frog *Leptobrachella tamdil* at its type locality. The places occupied by *L. tamdil* in the wild are characterised by 13–25 °C night temperature, 10–20 °C water temperature, 54–86% humidity, 6.84–7.15 pH, and 5–7 mg/L dissolved oxygen. We observed eight orders of prey in the gut contents of *L. tamdil*, examined by stomach-flushing live frogs among which the order Diptera was the most abundant, followed by Hymenoptera, while Hemiptera and Coleoptera were the least abundant.

Keywords: Anuran, biodiversity hotspot, Chhawl-chang, food, gut contents, Indo-Burma, microhabitat, natural history.

Mizo: Tamdil Chhawl-chang (*L. tamdil*) khawsak phung leh an chaw ei zirchianna Tamdil-ah neih a ni a. Kan zir hun chhung hian zan lam boruak chu 13–25° C, tui vawh zawng 10–20° C, boruak uap zawng 54–86% a ni. Heng uchang kawchhung atanga rannung chi hrang hrang order chi 8 rhuh a ni a, chung zingah chuan Diptera (tho/thosi lam chi) an tam ber a, Hymenoptera (fanghmir lam chi) ten an dawt a, chutih lain Hemiptera (khuangbai lam chi) leh Coleoptera (Rawmung lam chi) te an tlem ber a ni.

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INTRODUCTION

Understanding the habitat selection of an organism is essential in determining its distribution and behaviour. For this, we need to understand the behavioural interaction with its surrounding environments and quality of habitats (Figuera & Crowder 2006). Amphibians begin their lives in water, and terrestrial dwellers later transition to moist terrestrial habitats (Wells 2007). They remain closely linked to specific microhabitats and their precincts. Amphibians are endothermic animals, and are sensitive to rising temperatures. They depend on an external source to raise body temperature by absorbing solar radiation (Carey 1978). Climate change is one factor threatening amphibian populations, driving them into decline (Stuart et al. 2004). Amphibian assemblages in natural forests are always higher than in areas disturbed by anthropogenic activities, viz., jhum (slash-&-burn) cultivation land, plantation, and logging (Pawar 1999; Krishnamurthy 2003), and the presence of leaf litter and canopy cover are predictors for their assemblage (Balaji et al. 2013).

Leaf litter presence and depth are essential for the occurrence of amphibians (Fauth et al. 1989; Allmon 1991). Vegetation, sunlight, temperature, and other environmental factors strongly influence the distribution and activities of amphibians (Halverson et al. 2003). The ecological organisation of a community and its assemblage, the phylogenetic relationship among species, their behaviour, and their physiology can all be used to understand to a large extent the species' feeding ecology (Lima & Magnuson 1998; Grant et al. 2006; Arroyo et al. 2008). The diet of amphibians may depend on seasonal availability of prey, presence of competitors, and other relevant factors (Isaach & Barg 2002). Amphibians also play an important role in the biological control of mosquitoes to control diseases like malaria and dengue (Raghavendra et al. 2008; Bowatte et al. 2013).

The megophryiid frog genus *Leptobrachella* Smith, 1925 consists of 104 species, out of which four species, viz., *L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010), *L. lateralis* (Anderson, 1971), *L. nokrekensis* (Matthew & Sen, 2010), and *L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010), are found in India, all restricted to the northeast of the country (Frost 2025). The Tamdil Leaf-litter Frog (*L. tamdil*) was described from Tamdil wetland, Saitual District, Mizoram, in 2010 (Sengupta et al. 2010). Later on, additional records were made from Dampa Tiger Reserve, Hmuifang Community Reserve Forest, Sizhau Lake from Zotlang, Champhai District (Vanlalsiammawii et al. 2020; Muansanga et al. 2021) and also from Manipur (Decemson et al. 2021). However, the natural history of

the frog is still largely unknown (Vanlalsiammawii et al. 2020). Muansanga et al. (2021) partially reported the diet of *L. tamdil* to be insects of four orders based on the study of three individuals of *L. tamdil*. Herein, we present the habitat association and diet of *L. tamdil* studied at the type locality, based on in-situ field observations of wild frogs.

METHODS

Study area

Tamdil wetland was enlisted as a National Wetland in 2006–2007 by the National Wetland Conservation Programme, Government of India. It is located in the Saitual District of Mizoram (23.71° E & 92.95° N), about 100 km from the capital district, Aizawl and it covers about 13.7 km² with a mean elevation of 760 m (Image 1). The area falls under the Indo-Burma biodiversity hotspot and the vegetation type falls under Cachar Tropical Semi-evergreen Forest (2B/2C) category of Champion & Seth (1968).

Data Collection

Studies were conducted from February 2023 to May 2024 along the streams in Tamdil Wetland. The species were found to be active during the pre-monsoon months before the onset of peak monsoon. Visual encounter survey (Crump & Scott 1994), audio encounter survey, and opportunistic search were deployed to assess the diet during the survey period. Microhabitats in which the individuals were found are recorded along with relative humidity and temperature. The ecological parameters were recorded in the field using portable devices. We photographed the targeted species in its natural habitat with minimal disturbances.

Species confirmation

Genomic DNA was extracted from the collected tissue samples of one tadpole and one adult using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. A partial fragment (~570 base pairs) of the 16S rRNA was amplified and sequenced using previously published primers in Simon et al. (1994). Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95 °C for 3 minutes, followed by 39 cycles of denaturation at 94 °C for 45 seconds, annealing at 52 °C for 45 seconds, and extension at 72 °C for 2 minutes. Final extension was at 72 °C for 10 minutes. Amplified PCR products were run on a 2% agarose gel and viewed under UV transilluminator. Purified PCR product was sequenced

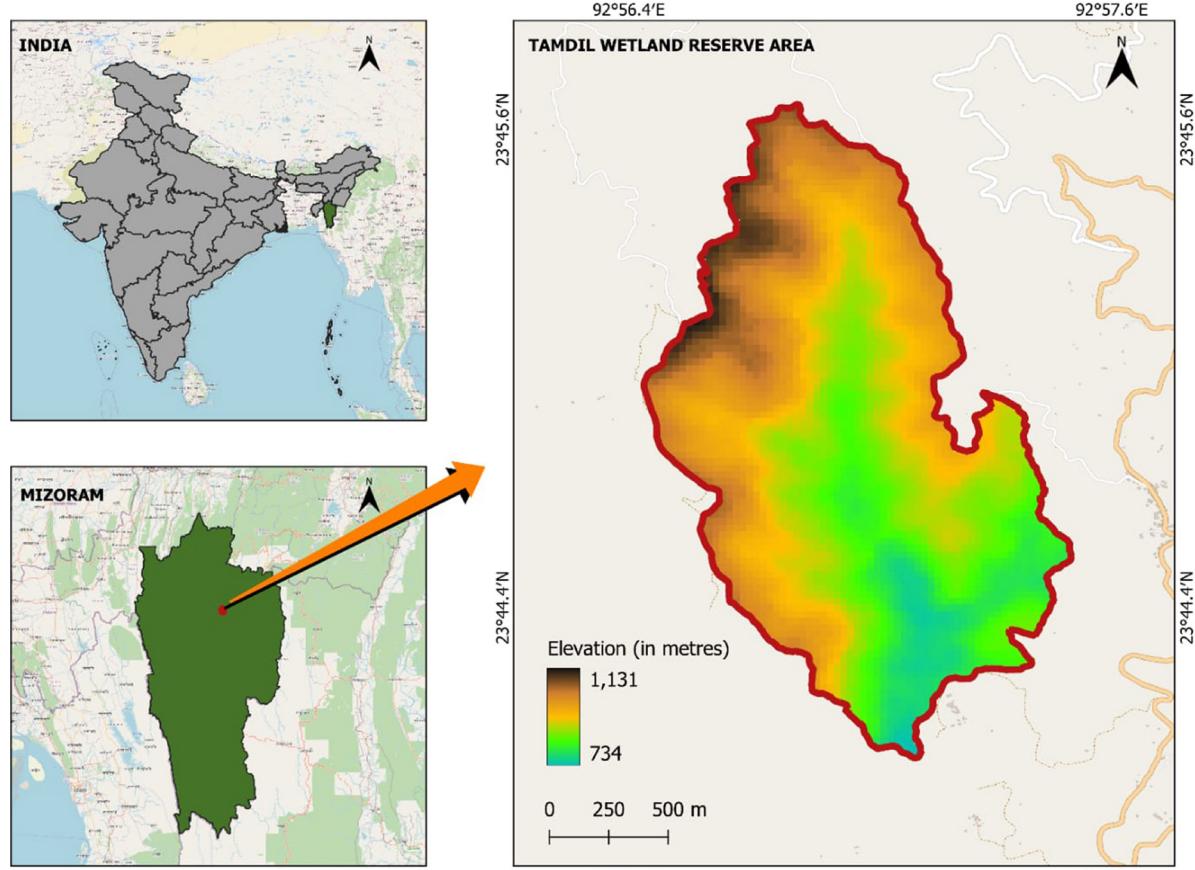


Image 1. Map of Tamdil Wetland (Inset: Elevation map), in Mizoram, northeastern India.

directly in an Applied Biosystems Genetic Analyzer 3500 XL in both directions using BigDye v3.1. The tadpole we studied was identified as *Leptobrachella tamdil* based on genetic congruence (0.11% in 16S rRNA) of our sample's sequence with published (also see Vanlalsammawil et al. 2020; Decemson et al. 2021; Muansanga et al. 2021) sequences of *L. tamdil* in GenBank (NCBI # ON500517.1, generated from MZMU 2675; ncbi.nlm.nih.gov/nuccore/ON500517.1).

Gut content analysis

Specimens were collected by hand and gut contents of the collected specimens were obtained by flushing the stomach using standardised protocol (Solé et al. 2005). The flushed contents are fixed in 10% formalin solution for laboratory analysis. The snout-vent length (SVL) of the specimens were taken using a calliper to the nearest 0.1 mm and they were released on the spot. The flushed gut contents obtained from the field were analysed in the laboratory using a Motic SMZ - 161 microscope and identified to the level of order. The abundance of various prey was estimated from the stomach contents

(percentage of the total number of individual prey/total number of all prey). Frequency of occurrence was determined by dividing the number of stomachs that contained a particular prey by the total number of stomachs with prey (Hyslops 1980). The rate of feeding activity was estimated as the percentage of stomach containing food with respect to the total number of stomachs examined (Sala & Ballesteros 1997). Rate of feeding activity = $100 n/N$ (where n is the number of stomachs with food, N is the total number of stomachs examined). Diet diversity was estimated using the Shanon-Weiner diversity Index (H), where $H = -\sum P_i \ln P_i$ and P_i = proportion of each taxa in the sample.

Food availability of the study area

The habitat where the species were most frequently encountered was selected for food availability estimation. Prey availability was estimated by making 10 plots of 1×1 m in the stream bank and inside the stream. The different types of potential prey (such as insects, spiders) were identified to the level of order and are left in the habitat. The potential preys were then compared with the actual

food items obtained from the gut of adult frogs.

RESULTS

We encountered different stages of *Leptobrachella tamdil* during the dry months when there is little precipitation and low stream flow (Image 2). Adults of *L. tamdil* were encountered under rocks, above rocks, in water-logged puddles, and on fallen leaves and twigs in and around the streambed. Encounters of tadpoles were usually in small water puddles, but upon approach, they bury themselves in mud quickly. The surrounding vegetation includes *Melocanna baccifera*, *Laurocerasus jenkinsii*, *Ficus fistulosa*, *Terminalia myriocarpa*, *Duabanga grandiflora*, *Macropanax* sp., *Pilea symmeria*, *Leea compactiflora*, *Drypetes indica*, *Aglaia spectabilis*, *Homalium ceylanicum*, *Phrynum capitatum*, and *Ensete* sp. (Image 3).

The air temperature during the study ranged from 13–25 °C at night; the water temperature ranged from 10–20 °C; the relative humidity ranged 54–86%; the pH of the water ranged 6.84–7.15; the dissolved oxygen ranged 5–7 mg/L. The species emerges mainly during the dry pre-monsoon months (January–May) when the water level is low, and pockets of small, waterlogged pools are formed in the depressions of the streambanks.

A total of 50 adult individuals of *L. tamdil* (SVL 25.2–35.9 mm) were examined for diet content. We obtained

Table 1. Prey availability of the Tamdil Leaf-litter Frog in the study area.

Hymenoptera	Ants
Orthoptera	Grasshoppers & crickets
Lepidoptera	Moths & butterflies
Hemiptera	Water striders, water bugs, & leafhopper
Isoptera	Termites
Diptera	Flies
Araneae	Spiders
Coleoptera	Beetles
Odonata (larvae)	Dragonflies & damselflies

81 prey items from 44 frogs, belonging to eight orders, viz., Hymenoptera (ants), Orthoptera (grasshoppers, crickets), Lepidoptera (moths), Hemiptera (leafhoppers), Isoptera (termites), Araneae (spiders), Diptera (flies), Coleoptera (beetles), and unidentified objects (Image 4). The remaining six individuals had empty stomachs. The rate of feeding was found to be 88% and the diversity of prey, measured with Shanon-Weiner diversity Index, is 1.90.

The order Diptera (28.40%) was the most abundant food item followed by Hymenoptera (20.99%). The least consumed food items were Coleoptera and Hemiptera (both at 2.47%) (Image 4). The frequency of occurrence was also calculated and it shows that Diptera (52.27%) and Hymenoptera (38.64%) were the most frequent

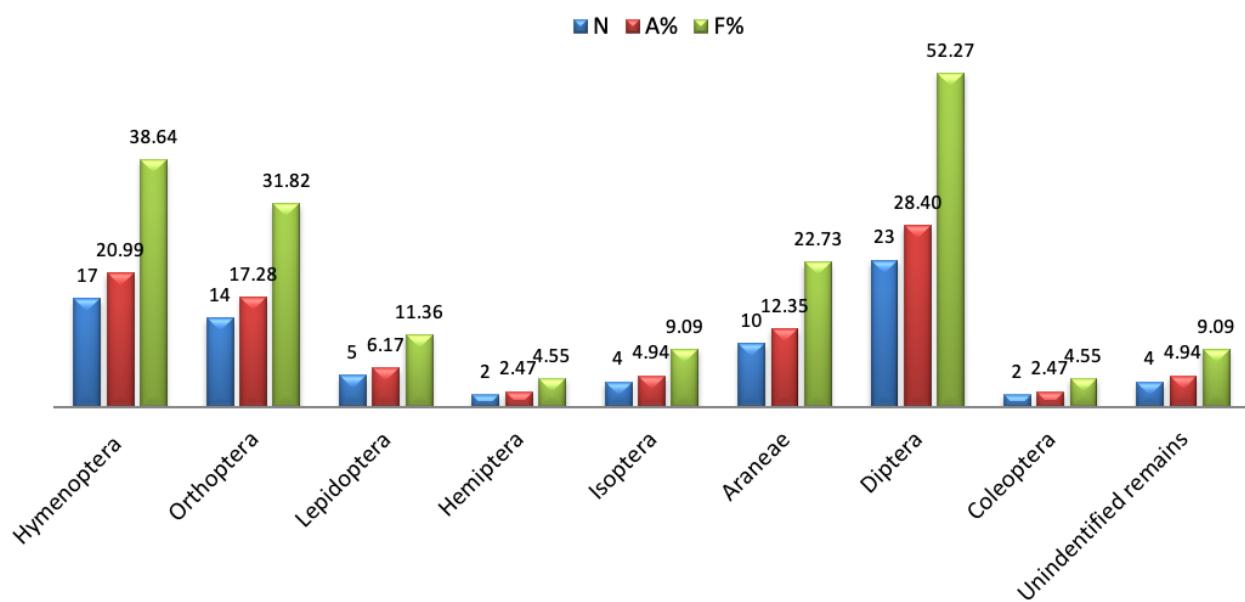


Figure 1. Frequency of occurrence and abundance of prey (%) in gut contents of *Leptobrachella tamdil*.



Image 2. Larvae, subadults, and adult *Leptobrachella tamdil* in its microhabitat. © Malsawmdawngliana.



Image 3. Habitat of *Leptobrachella tamdil* at the type locality – Tamdil wetland (Images A–D indicate different parts of the stream banks in the study area). © Malsawmdawngliana.



Image 4. Diet of *Leptobrachella tamdil*: A—Hymenoptera | B—Orthoptera | C—Lepidoptera | D—Hemiptera | E—Isoptera | F—Araneae | G—Diptera | H—Coleoptera | I—Unidentified objects. © Malsawmdawngliana.

contents (Figure 1). We recorded nine orders of potential prey species during the study (Table 1).

DISCUSSION

After the description of *L. tamdil* based on two individuals by Sengupta et al. (2010), there were no reports of this species for almost a decade. Vanlalsiammawii et al. (2020) recorded the third individual from Dampa Tiger Reserve (~54 km from type locality) and subsequent records were made from Hmuifang Community Reserve Forest (~39 km from type locality), Zotlang (~52 km from type locality), and Chakpi stream (~122 km from type locality) (Decemson et al. 2021; Muansanga et al. 2021). These, however, are inventory studies that were at the alpha taxonomic level, and the information on the natural history and its ecology is still at its infant stage. This study found that *Leptobrachella tamdil* inhabits forest floors and hill streams with rocks, similar to reports made in other studies on its congeners, viz., Lathrop et al. (1997)

(*L. ailaonicum*, *L. sungi*), Matsui (2006) (*L. fuliginosa*), Jiang et al. (2013) (*L. zhangyapingi*), Rowley et al. (2013) (*L. botsfordi*), and Tron et al. (2015) (*L. khasiorum*).

This study found eight orders of prey in the gut of *L. tamdil* of which the most abundant prey were Diptera and Hymenoptera, and also the most frequent food items obtained. Muansanga et al. (2021) reported four orders of insects, i.e., Orthoptera, Hemiptera, Hymenoptera, and Coleoptera from the gut contents of three individuals of *L. tamdil*. The gut contents observed by Muansanga et al. (2021) were also found in our studies with the addition of Lepidoptera, Isoptera, Araneae, and Diptera. Although studies have recorded that body size and prey size have a positive relation in amphibians (Quigora et al. 2009), *L. tamdil* is a medium-sized frog species, and the size of male and female individuals do not vary much, as deduced during our study.

Available prey of *L. tamdil* is represented in Table 1, and most of the available food items (except for Odonata) were found in the gut of the examined frogs. We cannot rule out the possibility that *L. tamdil* feeds on odonates, as

most amphibians are opportunistic feeders. The present study demonstrated that the diet of *L. tamdil* is largely composed of the available food items in their habitat. The peak active season of the frog was before the onset of the monsoon (Sengupta et al. 2010; Vanlalsiammawii et al. 2020; Decemson et al. 2021; Muansanga et al. 2021) when insect activity is low, which may influence the availability of potential food in the area. Additionally, plants, vegetation remains, sand, rocks, and other particles may be accidentally ingested and are therefore not counted as part of the diet. We have a few caveats in our study as this is the first attempt to study the ecology and natural history of *L. tamdil*. This study will, nevertheless, further help in the conservation of this endemic species and documenting its natural history.

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