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Assessing Reproductive Ecology and Oviposition Habitat Selection Among Anurans in Ayer Hitam Forest Reserve, Puchong, Selangor, Malaysia

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ABSTRACT

This study explored the breeding ecology and oviposition site selection of anurans in Compartment 15 of Ayer Hitam Forest Reserve (AHFR), focusing on the relationship between habitat variables and oviposition sites. The effectiveness of artificial breeding ponds was also assessed. Ten breeding ponds (five natural, five artificial) were studied from June to November 2022. Frog sampling occurred twice weekly at night, and tadpoles were observed weekly. Microclimate, macrohabitat data, and anuran presence were recorded. A total of 67 anurans from 18 species and six families were documented. *Hylarana labialis* had the highest number of individuals (12) near natural ponds, while *Kalophrynus palmatissimus* had the same number across natural and artificial ponds. The latter species, endemic to the region, is classified as Endangered (EN) by the IUCN Red List Index 2024. Tadpoles of *H. labialis*, *K. palmatissimus*, and *Microhyla* sp. were also documented. The study provides valuable insights into anuran habitat selection and microclimate influences. While these species show adaptability in disturbed areas, further research is needed to understand the impact of forest disturbance on their breeding ecology, as habitat loss could affect their populations.

Keywords: Artificial breeding ponds, conservation, endangered (EN) species, microclimate, macrohabitat

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INTRODUCTION

The success of anuran reproduction is heavily influenced by the selection of suitable breeding sites (Buxton et al., 2016), which directly impacts larval survival, development, and overall population dynamics (Rudolf & Rödel, 2005). Numerous studies have explored the habitats and ecology of anuran

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species; however, a significant gap remains in our understanding of artificial breeding ponds in Malaysia. This study seeks to address this gap by focusing on the conservation of endangered anuran species within the Ayer Hitam Forest Reserve (AHFR) in Puchong, Selangor. Specifically, we aim to provide insights into the breeding ecology and oviposition site selection of anurans, with a particular emphasis on endangered species in AHFR.

The selection of oviposition sites has a direct impact on biological fitness and is influenced by numerous environmental factors, which include predator presence, water temperature, risk of desiccation, the substrate for laying eggs, and the chemical characteristics of the water body (Sánchez-Ochoa et al., 2020). Oviposition site selection in anurans is influenced by abiotic and biotic factors, with parents often choosing sheltered locations that retain moisture and provide cover to protect eggs from desiccation and predation, while factors like nest moisture, pH, proximity to water, and predator presence play key roles in determining site quality (Fischer, 2023). The relative importance of a single factor might depend on the impact of other habitat parameters, making the selection of oviposition sites context-dependent (Fischer, 2023; Reich & Downes, 2003). Additionally, tropical rainforests are highly dynamic ecosystems where the availability and quality of suitable breeding habitats can fluctuate unpredictably (Peigner et al., 2023). In regions like AHFR, where natural water bodies may be limited or degraded, artificial ponds could serve as essential breeding habitats for amphibians, thereby playing a crucial role in their conservation (Brand et al., 2010).

Few studies consider multiple selective forces on oviposition site selection, which limits our understanding of the relative impact of different processes on this critical life history trait and hinders the development of effective conservation strategies for frogs (Sánchez-Ochoa et al., 2020). To address this, our research investigates the factors influencing anuran oviposition site selection within AHFR, focusing on both natural and artificial breeding sites. This involves documenting the anuran species present near these breeding ponds and assessing the effectiveness of artificial ponds in supporting anuran reproduction. By identifying key microclimate and macroclimate parameters that influence habitat selection, this study will contribute to a better understanding of how artificial breeding ponds can support anuran populations, particularly those that are endangered.

Amphibians are among the most threatened vertebrates, facing significant extinction risks due to habitat loss, diseases, and over-exploitation (Bates et al., 2019; Luedtke et al., 2023; Whittaker et al., 2013). The Global Amphibian Assessment (GAA2) on the IUCN Red List highlights that 41% of amphibian species are experiencing enigmatic declines, with habitat loss being a primary concern, especially in agricultural and urbanized regions (Luedtke et al., 2023). In such areas, artificial ponds may serve as the only viable breeding habitats for amphibians, underscoring their potential importance in conservation efforts (Brand et al., 2010).

Thus, while we have a good understanding of how ovipositing organisms respond to single factors, we still know little about how these responses translate into more complex situations with multiple factors. Identifying the factors influencing oviposition site selection in amphibians is crucial for the conservation of threatened, geographically restricted, and rare species. Thus, while we have a good understanding of how ovipositing organisms respond to single factors, we still know little about how these responses translate into more complex situations with multiple factors. Identifying the factors influencing oviposition site selection in amphibians is crucial for the conservation of threatened, geographically restricted, and rare species. This study seeks to address the question: What are the factors influencing oviposition site selection in anurans, and how effective are artificial breeding ponds in supporting anuran reproduction within AHFR? The findings will not only inform future research but also guide conservation strategies aimed at preserving amphibian populations in the tropical forest of Malaysia and similar ecosystems worldwide.

METHODS

Study Area and Sampling Sites

In this study, sampling was conducted in Compartment 15 of AHFR, Puchong, Selangor, focusing on two types of breeding ponds: natural and artificial. The geographic coordinates of each site were recorded using a Garmin GPSMAP 64S Handheld device. Environmental data were systematically documented, including the geographic location of each pond, the density of surrounding vegetation, light intensity, ambient temperature, microhabitat structures within the ponds, and the dimensions (size and depth) of the ponds.

For artificial breeding sites, black trays measuring 98 cm × 67 cm × 9 cm were utilized as experimental ponds. Initially, these trays contained only rainwater, and no substrate (e.g., leaves, sand, or stones) was introduced manually; instead, substrates accumulated naturally over time.

Anuran Sampling and Environmental Monitoring

Anuran populations were sampled within a 20-meter radius of the breeding ponds across all 10 sites. The sampling frequency was increased to twice a week (Friday and Saturday nights) from July 2022 to November 2022, as opposed to the once-every-three-weeks schedule used in previous studies (e.g., Caballero-Díaz et al., 2022). Sampling was conducted in diverse habitats, including swampy areas, trails, and stream edges. Captured anurans were temporarily contained in ventilated spherical plastic containers (Hidayu, 2019; Nadia et al., 2020).

During sampling, various microhabitat structures and environmental parameters were recorded. Microclimate data, including environmental temperature (°C), light intensity (lux), wind speed (mph), and humidity (%), were monitored using a Lutron-LM-8010 anemometer (Nadia et al., 2020). Soil pH was measured with a Takemura DM-15 pH soil instrument (Faris, 2019), while water parameters, including temperature (°C), pH, and dissolved oxygen levels (mg/L), were assessed using a Myron L UltraPen PT2 pH meter and a BLE-9100 dissolved oxygen analyser, respectively.

Data Collection and Analysis

Comprehensive data collection included measurements of egg clutches, egg sizes, and larval stages within the breeding ponds. Microorganisms present in the ponds were collected and examined under an Olympus microscope in a laboratory setting. Adult anurans encountered in the sampling areas were captured for sex determination and body measurements, which included weight, snout-vent length (SVL), head length (HL), head width (HW), snout length (SL), tibia length, and hindlimb length, all measured with a vernier caliper (Gvoždík et al., 2008; Nadia et al., 2020).

Captured anurans were transported to the AHFR laboratory for species identification. High-resolution photographs of each specimen were taken from dorsal, lateral, and ventral perspectives. Identification was conducted using Norhayati Ahmad's (2017) reference book 'Frogs and Toads of Malaysia' and the online database 'Ecology Asia' (www.ecologyasia.com). To prevent repeated sampling of the same individuals, each anuran's foot was marked with non-toxic nail polish before they were released back into their original habitat.

Statistical Analysis

Species diversity was quantified using the Shannon-Weiner diversity index (H), which accounts for both species richness and the relative abundance of each species within the community (Kassie et al., 2023). Species evenness, representing the uniformity of species abundances, was calculated using Pielou's evenness index. Data were analysed using Paleontological Statistics (PAST) software Version 3.18. The Shannon-Weiner Diversity Index was calculated using Equation 1:

$$\boldsymbol{H'} = -\sum_{j=1}^{S} \rho_i \ln \rho_i$$

$$\boldsymbol{E} = \mathrm{H}/\ln(\mathrm{S}) \qquad [1]$$

Where ρ_i = proportional abundance, H' = Diversity Index, E = evenness index, and S = the total number of species

All data were recorded in Microsoft Excel and analysed using IBM SPSS Statistics 27. An independent t-test was employed to assess significant differences between natural and artificial breeding ponds in terms of microclimate (Kim, 2015). Pearson's correlation coefficient (r) was utilized to examine relationships between oviposition site selection and various habitat variables (Hazra & Gogtay, 2016; Yadav, 2018). Additionally, a Kruskal-

Wallis test was applied for overall comparisons between anuran species and study areas (McKight & Najab, 2010), and a Spearman's Correlation Coefficient test was conducted to explore correlations between anuran species and microclimate variables (Zar, 2005). This approach ensures rigorous data collection and analysis, facilitating a comprehensive understanding of anuran diversity and the factors influencing their breeding ecology.

RESULTS AND DISCUSSION

Anuran Species Recorded in Ayer Hitam Forest Reserve, Puchong

Sampling within a 20-meter radius of each breeding pond, including nearby trails, streams, and swampy terrain, in Compartment 15 of AHFR, Selangor, recorded 67 individuals from 18 species across six families (Table 1). Newly recorded species included *Kurixalus chaseni* and *Nyctixalus pictus*. According to Hazieq (2023), AHFR harbors approximately 39% of the region's amphibian species, identifying 43 species from six families from 1975 to 2023. Since 2019, three newly recorded species have been documented, including the two from this study.

Table 1
The snout-vent length and sex of anuran records

No	Family	Species	SVL range (mm)	SVL mean ± SD/SE (mm)	Sex	
					M	F
1.	**Bufonidae**	*Duttaphrynus melanostictus*	73.0	73.0	1	0
2.		*Ingerophrynus quadriporcatus*	31.0	31.0	1	0
3.	**Dicroglossidae**	*Fejervarya cancrivora*	35.0 –73.0	57.7 ± 18.1/9.1	1	2
4.		*Limnonectes blythii*	30.0 – 103.0	66.5 ± 51.6/36.5	1	1
5.		*Limnonectes malesianus*	35.0 – 102.0	55.3 ± 23.9/9.0	4	2
6.		*Occidozyga laevis*	24.0 – 39.0	33.5 ± 6.7/2.7	6	1
7.	**Megophrynidae**	*Leptobrachium nigrops*	45.0	45.0 ± 0.0	0	2
8.	**Microhylidae**	*Kalophrynus palmatissimus*	34.0 – 43.0	38.1 ± 3.5/1.0	8	4
9.		*Microhyla berdmorei*	21.0 – 28.0	24.5 ± 4.9/3.5	1	1
10.		*Microhyla mantheyi*	26.0	26.0	1	0
11.	**Ranidae**	*Hylarana glandulosa*	36 – 92	69.5 ± 23.7/11.9	0	4
12.		*Hylarana labialis*	16.0 – 48.0	35.8 ± 10.6/4.3	7	5
13.		*Hylarana laterimaculata*	43.0	43.0	0	1
14.		*Hylarana nicobariensis*	37.0 – 45.0	41.8 ± 4.0/1.8	2	3
15.	**Rhacophoridae**	*Kurixalus chaseni*	34.0 – 36.0	35.0 ± 1.4/1.0	1	2
16.		*Nyctixalus pictus*	35.0	35.0	0	1
17.		*Polypedates leucomystax*	43.0	43.0	0	1
18.		*Rhacophorus pardalis*	45.0 – 57.0	53.0 ± 6.9/4.0	2	1
Total			-	-	**36**	**31**
Total no. of individuals				**67**		

Note. SVL = snout-vent length; F = female; M = male

The relatively low number of anuran individuals recorded in this study (67 individuals) may be attributed to several ecological and methodological factors. Amphibian populations are highly influenced by seasonal variations and climatic conditions, particularly rainfall and temperature, which affect breeding activity and calling behaviour (Mehra et al., 2021).

Ayer Hitam Forest Reserve (AHFR) has undergone significant habitat modification due to historical logging and ongoing anthropogenic activities. Compartment 15, in particular, is situated near a developed area featuring visitor facilities and a gravel road and has been designated as a development and demonstration zone (Abdullah et al., 1999; Sa'adah, 2018). Such modifications can reduce habitat connectivity, alter microclimatic conditions, and negatively impact anuran populations, particularly ground-dwelling species (Püttker et al., 2020; Ramalho et al., 2022). Comparatively, Faris (2016) also recorded a low number of 45 individuals from the same compartment.

Of the total, 51 individuals from 16 species across six families were recorded at natural ponds, while artificial ponds hosted 16 individuals from nine species across four families (Table 2). Natural ponds had the highest abundance of Ranidae (19), followed by Dicroglossidae (13) and Microhylidae (10). In artificial ponds, Dicroglossidae and

Table 2
Number and species of anurans in two types of breeding ponds

No	Family	Species	No. of Individuals (n)		
			Natural Pond	Artificial Pond	Total
1.	**Bufonidae**	*Duttaphrynus melanostictus*	0	1	1
2.		*Ingerophrynus quadriporcatus*	1	0	1
3.	**Dicroglossidae**	*Fejervarya cancrivora*	2	1	3
4.		*Limnonectes blythii*	1	1	2
5.		*Limnonectes malesianus*	3	3	6
6.		*Occidozyga laevis*	5	2	7
7.	**Megophrynidae**	*Leptobrachium nigrops*	2	0	2
8.	**Microhylidae**	*Kalophrynus palmatissimus*	7	5	12
9.		*Microhyla berdmorei*	2	0	2
10.		*Microhyla mantheyi*	1	0	1
11.	**Ranidae**	*Hylarana glandulosa*	3	1	4
12.		*Hylarana labialis*	12	0	12
13.		*Hylarana laterimaculata*	0	1	1
14.		*Hylarana nicobariensis*	4	1	5
15.	**Rhacophoridae**	*Kurixalus chaseni*	3	0	3
16.		*Nyctixalus pictus*	1	0	1
17.		*Polypedates leucomystax*	1	0	1
18.		*Rhacophorus pardalis*	3	0	3
Total no. of individual			**51**	**16**	**67**
Total species (%)			**16 (89%)**	**9 (50%)**	**18**

Microhylidae were the most abundant (five each). Certain species, including *Ingerophrynus quadriporcatus*, *Leptobrachium nigrops*, and *Nyctixalus pictus*, were exclusive to natural ponds, while *Duttaphrynus melanostictus* and *Hylarana laterimaculata* were unique to artificial ponds.

Hylarana labialis had the highest number of individuals (12), predominantly found on Bertam tree leaves near natural ponds. Its absence near artificial ponds might be due to its location in open areas without nearby vegetation. This species, tolerant of habitat disturbances, is associated with forested streams, as reported by Bahiah et al. (2019) and the IUCN (2024). Similar findings have documented its presence on palm leaves, such as *Pandanus* and *Licuala grandis* (Inger & Stuebing, 2005).

Kalophrynus palmatissimus, another abundant species (12 individuals), was recorded across five ponds, including natural ponds (NP II, NP III) and artificial ponds (AP I, AP III, AP IV). Consistent with previous studies (Faris, 2019; Faris et al., 2021; Hidayu, 2019; Nadia, 2017; Norhayati, 2017; Sa'adah, 2018), this species prefers forest litter and open areas like trekking trails. Similarly, Badli-Sham (2023) reported its presence in open areas, such as camping sites and trails, at Sekayu Recreational Forest in Terengganu. Additionally, *Kalophrynus* species utilize temporary ponds and phytotelmata (e.g., root buttresses and hollow tree trunks) for breeding (Haas et al., 2022; Vassilieva & Nguyen, 2023).

Captured Anurans in Natural Pond (NP) and Artificial Pond (AP)

From Table 3, NP II had the highest number of captured anurans (19 individuals), followed by NP I (18). In contrast, NP V and AP II had no captures. NP III and NP IV each recorded seven individuals, while AP I and AP IV had five, AP V had four, and AP III had two. These variations highlight differences in anuran abundance across ponds, with NP II emerging as the most populated.

NP II, the largest pond surveyed (2000 m²), exhibited features conducive to anuran diversity, such as shallow depth (15–30 cm), proximity to trails (5–10 m), and light penetration, which can influence calling activity and reproductive success (Kobisk & Kwiatkowski, 2023; Touchon & Warkentin, 2008). Despite its size, NP II had the most acidic pH (6.22), supporting species adapted to such conditions. Pond area, light penetration, and water pH significantly influence amphibian detection probability and reproductive success (Feldman et al., 2023).

The absence of anurans in NP V and AP II was likely due to habitat disturbances and structural limitations. NP V, frequented by the local Orang Asli community, experiences human activity that may disrupt anuran populations (Aureo et al., 2019). AP II, located on steep terrain and surrounded by dense canopy cover (416.29 ± 253.02 lux), had limited light penetration and leaf litter accumulation, making it unsuitable for anurans (Sánchez-Ochoa et al., 2020).

Artificial ponds utilize black trays (98 cm × 67 cm × 9 cm), which pose minimal risk to amphibians compared to deeper artificial ponds, where mortality risks are higher due to trapping (Wei et al., 2023). Most artificial ponds monitored in this study pose medium to high risks due to persistent high water levels during rainy seasons.

In natural ponds, species such as *Kalophrynus palmatissimus*, *Leptobrachium nigrops*, *Microhyla berdmorei*, and *M. mantheyi* predominantly occupied the forest floor. In artificial ponds, anurans were concentrated in microhabitats such as forest litter and sandbanks, highlighting their habitat-specific preferences (Table 3).

Table 3
Number of anuran individuals caught at each pond

Pond	No. Individual (n)	No. of Species (%)
Natural pond (NP)		
NP I	18	6 (38%) *Hn, Hl, Iq, Lb, Ol, Hg*
NP II	19	9 (56%) *Hn, Kp, Lm, Ln, Mb, Mm, Ol, Hg, Rp*
NP III	7	3 (19%) *Kc, Kp, Hl*
NP IV	7	5 (31%) *Hn, Hl, Fc, Ln, Np*
NP V	0	0 (0%)
Artificial pond (AP)		
AP I	5	3 (33%) *Fc, Kp, Ol*
AP II	0	0 (0%)
AP III	2	2 (22%) *Kp, Lm*
AP IV	5	4 (44%) *An, Dm, Kp, Hl*
AP V	4	3 (33%) *Lb, Lm, Hg*

Note. *Dm: Duttaphrynus melanostictus, Fc: Fejervarya cancrivora, Hg: Hylarana glandulosa, Hl: Hylarana labialis, Hl: Hylarana laterimaculata, Hn: Hylarana nicobariensis, Iq: Ingerophrynus quadriporcatus, Kc: Kurixalus chaseni, Kp: Kalophrynus palmatissimus, Lb: Limnonectes blythii, Lm: Limnonectes malesianus, Ln: Leptobrachium nigrops, Mb: Microhyla berdmorei, Mm: Microhyla mantheyi, Np: Nyctixalus pictus, Ol: Occidozyga laevis, Pl: Polypedates leucomystax,* and *Rp: Rhacophorus pardalis*

Anuran Family and Their Habitat Preference

The study documented anuran species across various families, revealing distinct habitat preferences that reflect their ecological adaptability. The family Bufonidae included *Duttaphrynus melanostictus* and *Ingerophrynus quadriporcatus*. *D. melanostictus*, observed near disturbed landscapes and lowland regions, showed adaptability to bare soil and grass areas, with breeding occurring in artificial water bodies due to their ability to retain water, consistent with findings by Nadia (2020) and Hawkeswood and Sommung (2017). In contrast, *I. quadriporcatus* was associated with swamp forests and artificial habitats, such as plantations, relying on stagnant water for reproduction, which indicates vulnerability to habitat disturbances (Chan-ard et al., 1999; IUCN, 2024). The family Ranidae, represented by *Hylarana glandulosa*, *H. labialis*, *H. laterimaculata*, and *H. nicobariensis*, exhibited the highest species richness (22 individuals), predominantly near NP I, characterized by enclosed areas with palm trees. These ground-dwelling frogs adapt to a range of habitats but show a preference for forested plots, which provide necessary vegetation and microhabitats, as reported by Blackburn and Wake (2011) and Bahiah et al. (2019). Within the family Megophrynidae, *Leptobrachium nigrops* individuals were found near a natural breeding pond, highlighting their reliance on primary rainforest leaf litter and shallow forest streams for reproduction, with large, black tadpoles as a distinctive feature (Ecology Asia, 2024).

The family Microhylidae included *Kalophrynus palmatissimus*, *Microhyla berdmorei*, and *M. mantheyi*, totalling 15 individuals. *Kalophrynus palmatissimus* demonstrated exceptional camouflage and a preference for undisturbed lowland rainforests, while *Microhyla* species were associated with waterlogged grasslands and leaf litter habitats, emphasizing the importance of these microhabitats for their survival (Faris et al., 2019). The family Dicroglossidae comprised species such as *Fejervarya cancrivora*, *Limnonectes blythii*, and *Occidozyga laevis*, with 18 individuals recorded. These species showed habitat preferences ranging from marshy areas and small puddles to disturbed environments like plantations and forest trails (Jaafar et al., 2012; Klys, 2011). Notably, *O. laevis* thrives in aquatic microhabitats such as small streams and puddles, underscoring the critical role of water availability for breeding activities (Semlitsch & Bodie, 2003). The family Rhacophoridae, represented by *Kurixalus chaseni*, *Nyctixalus pictus*, *Polypedates leucomystax*, and *Rhacophorus pardalis*, included seven individuals. *Kurixalus chaseni* and *N. pictus*, newly identified in AHFR, preferred disturbed and primary forests (Gillespie et al., 2021). *Polypedates leucomystax* exhibited high adaptability to non-forested and restoration areas, often perching on shrubs and trees (Nadia et al., 2022), while *R. pardalis* demonstrated seasonal ground-level activity during reproduction, highlighting its primarily arboreal nature (Shahriza et al., 2011).

The study found higher diversity in natural breeding ponds ($H' = 2.47$, $S = 16$) compared to artificial ponds ($H' = 1.98$, $S = 9$) (Table 4), although evenness was comparable. Natural ponds support a wider range of species, including those less adaptable to artificial habitats. This aligns with Hazell et al. (2003), who emphasized the importance of conserving natural water bodies as they sustain species that rely on specific habitat conditions. These findings underscore the necessity of habitat conservation, particularly for anurans that depend on undisturbed ecosystems, to ensure the long-term survival of these ecologically significant species.

Table 4
Diversity indices of anurans

Parameter	Natural Breeding Pond	Artificial Breeding Pond
Total number of species (S)	16	9
Individuals (n)	51	16
Population mean (μ)	3.19	1.78
Shannon-Weiner index (H')	2.47	1.98
Evenness (E)	0.89	0.90

The diversity indices presented in Table 4 highlight a difference in species richness between natural and artificial ponds. The population mean (μ), representing the average number of individuals per species, is 3.19 in the natural breeding pond and 1.78 in the artificial pond, highlighting the superior ecological conditions of the natural habitat. Natural ponds, with their more complex and stable environments, offer a wider range of microhabitats and resources, supporting a greater diversity of anuran species compared to the simpler and more variable conditions of artificial ponds (Aureo et al., 2019; Feldman et al., 2023). In contrast, the artificial pond's lower population mean suggests reduced ecological suitability due to simplified structures and altered conditions, likely impacting species survival and reproduction. This disparity underscores the negative impacts of habitat modification on amphibian communities (Kabanze et al., 2024; Stuart et al., 2004). Conserving natural breeding habitats is essential for sustaining amphibian diversity and ensuring ecological stability.

Despite this difference in species richness, the evenness values for both types of ponds are high, approaching a value of one. This suggests a relatively uniform distribution of species abundance within each habitat, with no single species dominating the community. This reflects the stable ecological conditions maintained by both natural and artificial ponds, providing a balanced environment where each species can thrive without a significant competitive advantage or disadvantage (Touchon & Warkentin, 2008; Sánchez-Ochoa et al., 2020). Interestingly, the high evenness in the artificial pond may be due to the low species richness (S = 9) and total number of individuals (n = 16), which can lead to an artificial balancing effect where few individuals are distributed evenly among fewer species. Conversely, the natural pond, despite having a slightly lower evenness, supports a significantly higher number of species (S =16) and individuals (n =51), which may result in minor variations in species abundance, reflecting a more dynamic and competitive ecological setting. The data suggest that while the artificial pond appears to lack strong species dominance, this might be attributed to its reduced biodiversity and overall population size rather than ecological balance. In contrast, the natural pond's high species richness and moderate evenness indicate a more complex ecosystem where interspecies interactions and resource availability shape community structure.

Microclimate

This study examined nine microclimatic parameters: surrounding temperature (°C), air humidity (%), soil pH, soil moisture, wind speed (mph), light intensity (lux), water temperature (°C), water pH, and dissolved oxygen (mg/L). Due to nocturnal sampling in a forested area, light and wind consistently registered as zero. Table 5 presents differences in microclimatic conditions between natural and artificial ponds. Natural ponds had a slightly more acidic pH (mean: 6.62) compared to artificial ponds (mean: 7.00). Additionally, natural ponds exhibited higher water temperatures (27.00 °C) but cooler surrounding temperatures (29.70 °C) than artificial ponds (water temperature: 26.75 °C, surrounding temperature: 29.80 °C). Natural ponds also had higher dissolved oxygen and humidity levels (mean: 75.5%) compared to artificial ponds (mean: 73.2%). The natural pond area has lower light intensity (mean: 1512.5 lux) than artificial ponds (mean: 1646 lux). A t-test between the two pond types yielded a p-value below 0.05, indicating significant differences in their microclimates (Table 6).

Natural ponds generally offer more favourable environmental conditions for anuran species, including lower pH, higher water temperatures, cooler air temperatures, higher dissolved oxygen levels, increased humidity, and lower light intensity. These conditions are critical for anuran reproduction, as temperature, humidity, and light influence hormonal processes essential for breeding (Browne & Edwards, 2003). Rising temperatures, for example, can enhance feeding activity and metabolic processes, thus impacting frog abundance and productivity (Browne & Edwards, 2003; Carvalho-Rocha, 2020). Humidity is particularly crucial, as desiccation is a major threat to anuran survival. Species without parental care must select breeding sites with adequate humidity to avoid desiccation and ensure successful development (Angiolani-Larrea et al., 2024).

Soil moisture also plays a key role in amphibian survival, as moist conditions are necessary to maintain skin hydration for efficient gas exchange (Hoffmann et al., 2021). Studies show that higher soil moisture enhances frog survival, especially during the juvenile stage, and that damp conditions are sufficient for frog presence (Aryal et al., 2020; Haggerty et al., 2019). Additionally, correlation analysis revealed a significant relationship between humidity and the abundance of *Hylarana labialis* (p-value = 0.028), while no significant differences were found for *Kalophrynus palmatissimus* across other microclimate factors.

Ceron et al. (2020) highlighted that species composition within the Atlantic Forest's metacommunity fluctuates independently, influenced by seasonal temperature, rainfall, and humidity patterns. Similarly, Cicchino et al. (2020) found that air temperature and humidity impact water loss in frogs and frogs call more frequently when humidity and temperature are higher. These findings align with broader phenological patterns, emphasizing the importance of environmental cues in anuran reproductive success (Canavero et al., 2019; Chmura et al., 2019; Post, 2019).

In correlation analyses, environmental temperature showed significant differences with humidity (p-value = 0.003), dissolved oxygen (p-value = 0.011), water temperature (p-value = 0.005), and water pH (p-value = 0.041). Dissolved oxygen differed significantly from the other factors, except for light. Although *Hylarana labialis* and *Kalophrynus palmatissimus* showed correlations with environmental temperature, humidity, soil pH, and soil moisture, it is important to note that correlation does not imply causation, as other factors may influence the observed relationships (Gogtay & Thatte, 2017).

Table 5
The range and mean of microclimate data of the natural and artificial breeding ponds

Pond	Water pH	Water Temp. (°C)	Env. Temp. (°C)	DO (mg/L)	Soil pH	Light (lux)	Humidity (%)	Wind (mph)
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
NP I	6.62 ± 0.60	25.58 ± 0.71	29.35 ± 1.77	4.46 ± 1.93	6.00 ± 0.67	499.33 ± 234.39	78.62 ± 4.39	0.00 ± 0.00
NP II	6.22 ± 0.56	25.59 ± 0.72	29.48 ± 1.78	5.59 ± 2.24	6.51 ± 0.38	383.72 ± 188.45	78.47 ± 4.15	0.00 ± 0.00
NP III	6.42 ± 0.84	25.93 ± 0.98	29.51 ± 1.66	6.30 ± 4.39	6.10 ± 0.76	799.65 ± 636.80	77.33 ± 4.95	0.00 ± 0.00
NP IV	6.62 ± 0.65	26.01 ± 0.88	29.51 ± 1.59	6.72 ± 3.50	6.40 ± 0.36	383.47 ± 193.63	79.30 ± 3.81	0.00 ± 0.00
NP V	6.70 ± 0.68	26.04 ± 0.76	30.11 ± 1.72	6.20 ± 2.35	6.32 ± 0.21	984.73 ± 2.38	78.27 ± 4.17	0.00 ± 0.00
NP Mean	6.52 ± 0.19	25.83 ± 0.23	29.59 ± 0.30	5.86 ± 0.88	6.26 ± 0.21	610.18 ± 269.79	78.40 ± 0.71	0.00 ± 0.00
AP I	7.01 ± 0.67	26.48 ± 1.30	29.86 ± 1.62	6.49 ± 2.78	0.00 ± 0.00	1572.89 ± 893.65	79.10 ± 3.55	0.00 ± 0.00
AP II	7.08 ± 0.78	25.51 ± 0.80	29.89 ± 1.71	6.72 ± 3.50	0.00 ± 0.00	416.29 ± 253.02	75.90 ± 6.50	0.00 ± 0.00
AP III	7.14 ± 0.73	25.74 ± 0.87	29.94 ± 1.75	6.10 ± 2.41	0.00 ± 0.00	680.82 ± 468.56	76.49 ± 5.76	0.00 ± 0.00
AP IV	6.94 ± 0.65	25.89 ± 0.84	30.04 ± 1.55	6.49 ± 3.40	0.00 ± 0.00	541.47 ± 272.95	76.48 ± 5.27	0.00 ± 0.00
AP V	6.90 ± 0.67	26.05 ± 1.20	29.62 ± 1.71	6.92 ± 3.35	0.00 ± 0.00	177.12 ± 123.94	78.96 ± 3.80	0.00 ± 0.00
AP Mean	7.01 ± 0.10	25.94 ± 0.37	29.87 ± 0.15	6.54 ± 0.31	0.00 ± 0.00	677.72 ± 533.58	77.39 ± 1.52	0.00 ± 0.00

Note. Env: Environment, temp: Temperature, NP: Natural Pond, AP: Artificial Pond

Table 6
Comparative analysis of microclimate variables between natural and artificial breeding ponds with t-test results

	Mean Natural Pond	Mean Artificial Pond	t-value	df	*p*-value
Water pH	6.62 ± 2.38	7.00 ± 0.69	19.11	9	0.000
Water Temperature (°C)	27.00 ± 9.11	26.75 ± 1.06	55.88	9	0.000
Environment Temperature (°C)	29.70 ± 3.16	29.80 ± 1.65	78.67	9	0.000
Dissolved Oxygen (mg/L)	9.97 ± 3.41	8.16 ± 3.06	4.13	9	0.003
Light (lux)	1512.50 ± 462.10	1646.00 ± 650.12	2.69	9	0.025
Humidity (%)	75.50 ± 4.30	73.20 ± 5.19	31.36	9	0.000

Microhabitat

The study identified four primary microhabitats utilized by anurans: bare ground (49.51%), forest litter (28.16%), tree surfaces (11.65%), and pond or swamp areas (10.68%). Bare ground was the most frequently observed microhabitat, likely due to the terrestrial nature of most species recorded, belonging to families such as Bufonidae, Dicroglossidae, Microhylidae, and Megophrynidae. Arboreal species, such as those in Ranidae and Rhacophoridae, were less frequently captured, possibly due to their inherent elusiveness (Bahiah et al., 2019).

Forest litter was the second most utilized microhabitat, primarily associated with species from Megophrynidae and Microhylidae. Megophrynidae species, such as litter frogs, are well adapted to the forest floor, where their camouflage enhances survival (AmphibiaWeb, 2024). Similarly, the fossorial and terrestrial tendencies of Microhylidae, particularly *Kalophrynus palmatissimus* (12 individuals recorded), align with previous findings, highlighting their preference for litter-rich habitats (Badli-Sham et al., 2023; Nadia, 2017).

Forest litter provides critical ecological benefits, such as increased soil moisture conservation, shade, and stable humidity (Jourgholami et al., 2022), which are essential for species such as *Microhyla* sp. For example, *Microhyla annectens* species thrive in environments with 75%–95% litter coverage, conditions that help maintain hydration and activity levels under optimal temperature and humidity (Nadia et al., 2022).

Tree surfaces and pond/swamp areas were less frequently utilized, suggesting that they were either less suitable or less accessible for the majority of the recorded species. These findings reinforce the importance of maintaining diverse microhabitats to support anuran populations, particularly in light of habitat modification that could alter microhabitat availability and quality (Bahiah et al., 2019). Conservation strategies should prioritize the protection of heterogeneous microhabitats to preserve anuran biodiversity and ecological functionality.

Tadpole

Tadpole species observed in the ponds included *Hylarana labialis*, *Kalophrynus palmatissimus*, and *Microhyla mantheyi*. Tadpoles of *H. labialis* were recorded in natural ponds NP I and NP IV, while *K. palmatissimus* and *M. mantheyi* were observed in artificial pond AP I. Complete metamorphosis was documented for *H. labialis* and *K. palmatissimus* in their respective ponds. Interviews with forest officers revealed that *K. palmatissimus* primarily lays eggs in natural temporary ponds characterized by stagnant water and abundant leaf litter (Mohd Naeem Abdul Hafiz, personal communication, July 17, 2022). Interestingly, this species also utilized the artificial ponds during its larval stage.

Tadpoles were observed in two natural ponds (NP I and NP IV) and one artificial pond (AP I). Natural ponds, with sizes ranging from 120–2000 m^2, provided a more intricate habitat structure compared to the smaller artificial pond (0.66 m^2), which likely contributed to higher habitat diversity and more resources for the tadpoles. The larger area of natural ponds allowed for varying depths, substrates, and vegetation, which catered to the specific needs of different species (Hiragond & Saidapur, 2001). Additionally, the presence of leaf litter in natural ponds increases substrate complexity, moisture retention, and nutrient cycling, all of which are essential for tadpole development (Song et al., 2021). In line with these findings, Camacho-Rozo and Urbina-Cardona (2021) demonstrated that natural ponds support higher species richness, greater larval abundance, and more significant spatial and temporal turnover compared to anthropogenic water bodies, emphasizing the ecological advantages of natural habitats.

The natural ponds hosted protists, algae, and *Closterium* sp., forming a balanced food web for tadpoles. In contrast, artificial ponds contained mosquito larvae, dragonfly nymphs, and algae but had fewer microorganisms, thereby limiting nutritional resources. The presence of dragonfly nymphs, known predators of tadpoles, further heightened predation risks in artificial ponds (Kruger & Morin, 2020). Studies on tadpole diets indicate algae and protozoa are dominant food sources (Santos et al., 2016), suggesting that natural ponds may better support tadpole growth due to richer microbial diversity.

The artificial pond (AP I) hosted tadpoles of *Kalophrynus palmatissimus* and *Microhyla mantheyi*, indicating their adaptability to artificial habitats. However, natural ponds supported a single species, *Hylarana labialis*, likely due to their more stable environmental conditions and larger area, which reduces interspecific competition and predation risks. Tadpoles of *H. labialis* are generally larger compared to *K. palmatissimus* and *M. mantheyi*, possibly requiring a larger pond to support their developmental and survival needs. Furthermore, the constant water availability during the seasonal rainfall patterns, as observed in September, may synchronize anuran reproductive behaviours with pond availability and environmental stability, highlighting the critical role of climatic cues in amphibian life cycles (Canavero et al., 2019; Llusia et al., 2013;).

Leaf litter decomposition, driven by microbial activity and shredders, is a crucial ecological process for nutrient cycling and detritus formation in natural ponds (Iwai et al., 2009; Montaña et al., 2019). Tadpoles, as primary consumers, contribute significantly to the breakdown of organic matter, supporting nutrient cycling and aquatic food webs (Montaña et al., 2019). In contrast, the newly established artificial ponds lack the accumulated organic matter found in older natural ponds, which have had years of decomposition (Iwai et al., 2009). Additionally, the absence of key shredders, such as certain invertebrates and tadpole species, further hinders decomposition in these artificial environments.

Future studies should extend sampling periods to capture diverse breeding seasons and evaluate how habitat complexity, pond size, and predator-prey interactions influence the survival and development of tadpoles. Understanding these dynamics will aid in the conservation of anuran populations and their critical roles in freshwater ecosystems.

CONCLUSION

The natural breeding ponds demonstrate higher species diversity compared to artificial breeding ponds. *Kalophrynus palmatissimus*, an endemic and near-threatened species, exhibited the highest species abundance across both natural and artificial breeding ponds. The successful utilization of our artificial pond shows its potential to become an alternative breeding site for anurans in forested areas.

This research has been successful in benefiting the conservation of anuran species, particularly those classified as endangered or near threatened, across both breeding ponds. For future research, ensuring adequate depth of artificial ponds to prevent water overflow during heavy rains is critical. Expanding the sampling area and duration to encompass a broader range of anuran habitats and reproduction periods would also be advantageous. To enhance anuran populations, establishing additional artificial ponds is recommended to increase the availability of suitable oviposition sites. Increasing the number of these breeding habitats can promote greater anuran abundance and contribute to population resilience. This approach may mitigate the effects of habitat loss and fragmentation, supporting the long-term conservation of amphibian communities.

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