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ECOLOGICAL GUILDS OF ANURAN SPECIES FROM DIFFERENT ECOSYSTEM TYPES IN MALAYSIA, BORNEO (SARAWAK)

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Abstract: An ecological guild (ecological species concept) refers to species with similar ecological requirements adapted to a particular set of resources, called a niche, in the environment. Deforestation by severe clear cutting that leads to fragmentation of frogs' distribution, as well as overhunting for local usage, are the greatest threats to the animals, especially in Borneo. Comprehensive studies on frogs' ecological requirements are very much needed for a sustainable future. Hence, this study aims to document frog species compositions and determine the ecological guilds of frog species via non-metric multidimensional scaling. A total of 44 species from the families of Ranidae, Dicroglossidae, Rhacophoridae, Bufonidae, Megophryidae and Microhylidae were obtained. The ecological guilds of anuran species were clustered into a few significant groups, consisting of (i) ground-dwelling frogs of disturbed areas, (ii) arboreal frogs of disturbed areas, (iii) semi-arboreal (seedlings) frogs of disturbed areas, (iv) ground-dwelling frogs of forest areas, (v) arboreal frogs of forest areas and (vi) semi-arboreal (rock dwellers) frogs of forest areas. The findings suggest that diversity and distribution of frog species were influenced by the environment and selection of microhabitats. Frogs that share the same ecological requirements tend to co-exist. This study can provide insights into frog species documentation in a specific ecosystem, which is crucial for sustainability and conservation purposes.

Keywords: Assemblages, anurans, ecological guilds, microhabitat, sustainability.

Introduction

The concept of ecological guilds is commonly used for the plant kingdom, but rarely for animals as they have a high ability to move and adapt to various ecosystems. Changing environments are highly sensitive to amphibians due to their nature of having permeable skins (Zainudin *et al.,* 2018) and specific microhabitat requirements (Deka *et al.,* 2019; Zainudin *et al.,* 2017; Zainudin *et al.,* 2019). Thus, amphibians are one of the species affected by the major threats facing the world (Gillespie, 2012). Most frogs need water as the primary medium to breed, grow and survive (Inger *et al.,* 2017). Thus, good quality of water will provide a good living environment for the frog communities as the little changes in the water quality and microhabitat attributes can affect the frog population and this may lead to the increase in mortality rate of tadpoles or adult frogs. Microhabitats may also influence the frog population's behaviour and species diversity as different habitats provide different sources and services to their ecosystem (Moen & Wiens, 2017).

Ecological guilds and microhabitat utilisations of Bornean frogs have been previously studied (Gillespie *et al.,* 2012; Zainudin *et al.,* 2006; Zainudin *et al.,* 2017) but the details of species compositions and their assemblages are still inadequate. Moreover, the clustering of frogs based on ecological guilds may change over time since amphibians are good biological indicators of environmental changes. They can be selective of their habitats if there are any change in terms of specific resource utilisation due to environmental factors, evolutionary forces and threats (Warguez *et al.,* 2013; Zainudin *et al.,* 2017).

Frogs are susceptible organisms that can detect minor differences in their environment

(Zainudin *et al.,* 2019a). Even slight changes in the temperature and climate may affect the survival of the frog species in a particular habitat, as well as their assemblage patterns. Comprehensive studies on frogs' ecological requirements are very much needed for a sustainable future, especially in hotspot biodiversity areas. Hence, this study aims to document frog species compositions and determine the ecological guilds of frog species from selected ecosystems in Malaysian Borneo.

Materials and Methods

Sampling Localities

Three biogeographical regions were selected, comprising west, central and east Sarawak (Figure 1). Eastern Sarawak was represented by the Mulu National Park, a United Nations Educational, Scientific and Cultural Organisation (UNESCO) World Heritage Site, which promotes biodiversity-rich ecotourism with various vegetation types, flora and fauna. Bario, known as the Kelabit Highlands (Zainudin *et al.,* 2006) was also chosen as a site of study. Bario lies at approximately 1,200 m above sea level with Sungai Baram as its primary water source. It is surrounded by natural landscapes of forests, agriculture and native villages (Abdullah *et al.,* 2020).

Central Sarawak was represented by the Maludam National Park (peat swam ecosystem) and Batang Ai National Park (mixed dipterocarp ecosystem). The Maludam National Park is located in Betong Division, Sarawak, consisting of two main rivers, namely Batang Lupar and Saribas River. It covers an area of approximately 43,147 ha (Dosi *et al.,* 2018). The Batang Ai National Park, on the other hand, is located in Sri Aman Division and sits on the Batang Ai Dam surrounded by old and secondary rainforest. It also serves as the home to a variety of wildlife species, especially Kenyalang birds and the foot-flagging frog *Staurois laptopalmatus*.

Western Sarawak, on the other hand, was represented by the Tanjung Datu National Park, which is located at the tip of Sarawak and considered to be the smallest national park in the state, with a total area of approximately 1,379 ha (Mohd Azlan *et al.,* 2018). The Libiki Bamboo Resort is located in Bau, Sarawak, near the Malaysian-Indonesian national border and is

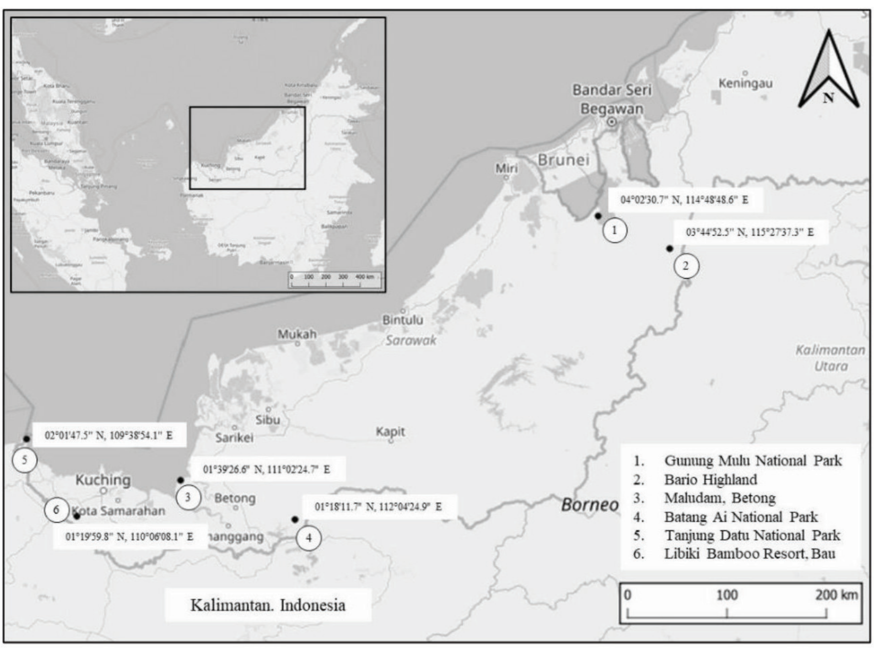

Figure 1: A map of the sampling localities in Sarawak

known for its ecotourism spot as it is surrounded by the *Kerangas* forest of Borneo.

Sample Collection

Forest transects by visual and auditory surveys were used when searching for the adult frogs (Afonso & Eterovick, 2007). The forest transects were performed in a three-hour duration (7.00 pm to 10.00 pm). All the samples were kept alive in plastics and brought back to the working bench. When capturing different species of anurans, it is important to note that they cannot be placed in the same container as this may cause deaths to some individuals due to the toxic secretion produced by some frogs as a self-defence mechanism (Rahman *et al*., 2008). *Odorrana hosii* is an example of a frog that can secrete poison when stressed, which may kill other frogs cause skin irritation in humans (Inger *et al.,* 2017; Rasit *et al.,* 2018).

The microhabitat data for each frog individual were recorded by following the habitat code of the Inger Habitat Code used by Heyer *et al.* (1994). Necessary measurements have also been taken, such as weight (W), total length (TL) and snout-vent length (SVL). The specimens were then fixed in 10% formalin and stored in 70% ethanol to be stored as voucher specimens. The samples were identified up until the species level by following the guides from Inger *et al*. (2017) and the Frogs of Borneo website (Haas *et al.,* 2019).

Data Analysis

Previous anuran collections from 2010 were included for data sampling. Non-metric multidimensional scaling (NMDS) analysis via IBM SPSS version 22.0 (IBM Corp, 2013) was used to determine the relationship between frog species and their microhabitat utilisation.

Results and Discussion

A total of 44 species from six genera were successfully obtained from all the sampling sites (Table 1). A total of 14 frog species were identified in Bario (number of individuals (N) = 48) and Tanjung Datu (N = 40), 13 in Libiki Bamboo Resort (N = 44), 11 in Mulu National Park (N = 23) and Batang Ai (N = 45) and 6 in Maludam, Betong (N = 46).

Approximately 45% of the overall species were dominated by the family Ranidae, followed by 26% from the family Dicroglossidae, 14% from the family Rhacophoridae, 12% by the family Bufonidae, 2% from the family Megophryidae and 1% from the family Microhylidae. Additionally, some of the species were endemic to Borneo such as *Limnonectes leporinus, Leptolalax dringi, Microhyla borneensis, Meristogenys whiteheadi* and *Philautus tectus* (Inger *et al.,* 2017).

Many of the frog species in the Sarawak region were classified as least-concern (LC) species in the IUCN Red List Category, 2020. Sadly, some have experienced a slow decline in population due to anthropogenic effects and threats. For example, *Meristogenys jerboa* is categorised as vulnerable (VU) whereas *Nyctixalus pictus* is near threatened (NT). The populations of *Meristogenys jerboa* and *Nyctixalus pictus* were considered to be threatened due to the fact that a smaller number of mature individuals have been found in recent years.

Non-metric multidimensional scaling (NMDS) through proximity scaling was derived in final coordinate dimensions (FCDs) 1 and 2 for each locality. The stress and fit measures (Table 2) shows that the stresses (Stress-1 = 0.175, Stress-2 = 0.279, S-stress = 0.063) and coefficient values (Tucker's coefficient of congruence = 1.000) indicate a significant representation of actual habitat and microhabitat dissimilarities.

Based on Table 3 and Figure 2, two types of vegetation were identified to have significant FCD values to describe the preferences of Sarawak frogs' ecological guilds. A total of 45 microhabitat variables were meaningful in describing habitat and microhabitat utilisation of Sarawak frog species. High positive loading value of FCD 1 = 1.599 was determined for the Primary Forest (VA) and FCD 2 = -1.075

Table 1: The complete list of frog species collected from six localities in Sarawak

Family	Species	Eastern Sarawak		Central Sarawak		Western Sarawak		IUCN Status
		Mulu (2016)	**Bario (2013)**	**Maludam (2020)**	**Batang Ai (2010)**	**Tg Datu (2019)**	**Libiki (2020)**	
Bufonidae	*Ansonia spinulifer**	-	-	-	-	5	1	LC
	Duttaphrynus melanostictus	-	-	2	-	-	-	LC
	Ingerophrynus divergens	-	-	-	-	-	1	LC
	*Pelophryne saravacensis**	-	-	-	-	2	-	NT
	Phrynoidis juxtaspera	1	5	-	12	-	-	LC
Dicroglossidae	*Fejervarya cancrivora*	-	-	-	-	1	-	LC
	Fejervarya limnocharis	-	-	-	-	-	4	LC
	*Limnonectes ibanorum**	2	1	-	5	-	-	LC
	*Limnonectes ingeri**	-	-	3	-	-	-	LC
	Limnonectes kong	-	-	-	-	9	-	-
	Limnonectes kuhlii	-	-	-	1	-	-	LC
	*Limnonectes leporinus**	2	2	-	7	-	-	LC
	Limnonectes malesianus	-	-	-	-	2	-	NT
	Limnonectes palavanensis	-	1	-	-	-	-	LC
	Limnonectes paramacrodon	-	-	15	-	7	2	NT
Megophryidae	*Leptolalax dringi**	-	-	-	-	-	2	-
	Megophrys nasuta	-	-	-	-	3	-	LC
Microhylidae	*Kalophyrnus meizon**	-	-	-	-	-	1	-
	*Microhyla borneensis**	-	-	-	-	-	1	LC
	*Microhyla malang**	1	-	-	-	-	-	LC

Ranidae	*Amnirana nicobariensis**	-	5	-	-	-	-	LC
	*Chalcorana megalonesa**	-	-	-	-	-	10	LC
	*Chalcorana raniceps**	-	-	8	7	1	11	LC
	Hylarana erythraea	-	6	11	-	-	-	LC
	*Meristogenys amoropalamus**	-	-	-	-	-	1	LC
	*Meristogenys dyscritus**	-	-	-	-	1	-	LC
	*Meristogenys jerboa**	-	-	-	-	2	1	VU
	*Meristogenys phaeomerus**	-	3	-	8	-	-	LC
	*Meristogenys whiteheadi**	-	-	-	1	-	-	LC
	Odorrana hosii	-	9	-	1	-	-	LC
	Pulchrana baramica	2	-	7	-	-	-	LC
	Pulchrana glandulosa	1	-	-	-	-	-	LC
	Pulchrana picturata	1	1	-	1	-	-	LC
	Pulchrana signata	-	1	-	-	-	-	LC
	*Staurois guttatus**	1	4	-	1	1	-	LC
	*Staurois latopalmatus**	2	-	-	1	-	-	LC
Rhacophoridae	*Kurixalus appendiculatus*	7	-	-	-	-	-	LC
	Nyctixalus pictus	-	-	-	-	1	-	NT
	Philautus petersi	-	-	-	-	3	-	LC
	*Philautus tectus**	-	-	-	-	2	-	LC
	Polypedates colletti	3	-	-	-	-	-	LC
	Polypedates leucomystax	-	2	-	-	-	8	LC
	Polypedates macrotis	-	7	-	-	-	-	LC
	*Polypedates otilophus**	-	-	-	-	-	1	LC
	Total individuals	23	47	46	45	40	44	
	Species captured	11	14	6	11	14	13	

*Species endemic to Borneo based on Inger *et al.* (2017)

Table 2: Stress and fit measures values of the NMDS configuration

Stress and Fit Measures	Values
Normalised Raw Stress	0
Stress-I	0.175a
Stress-II	0.279a
S-Stress	0.063b
Dispersion Accounted For (D.A.F.)	1.000
Tucker's Coefficient of Congruence (TCC)	1.000

PROXSCAL minimises Normalised Raw Stress

a. Optimal scaling factor = 1.024
b. Optimal scaling factor = 0.946

Table 3: Microhabitat variables of Sarawak frogs

No.	Microhabitats	Code	FCD 1	FCD 2
	Vegetation Types			
1	Primary rain forest, hilly	VA	1.599	1.432
2	Agriculture	Vagr	-0.804	0.263
3	Edge MDF	VE	-0.153	0.074
4	Secondary growth, immature/regeneration forest	VG	-0.062	0.041
5	Kerangas forest	VK	0.450	-1.075
6	Peat swamp	VW	-0.340	-0.962
	Horizontal Position			
7	Permanent stream: in water	HPA	-0.050	0.280
8	Permanent stream: midstream on bar, rock or snag	HPB	0.492	0.057
9	Permanent stream: on bank	HPC	1.768	-0.632
10	Intermittent stream: in water	HPD	-0.239	0.057
11	Intermittent stream: midstream on bar, rock or snag	HPE	-0.285	-0.067
12	Intermittent stream: on bank	HPF	-0.280	-0.297
13	Distant from any body of water	HPG	-0.583	0.833
14	Temporary pond, in water	HPJ	-0.460	0.139
15	Temporary pond, on vegetation	HPL	-0.327	0.362
16	Permanent pond, on bank	HPR	-0.268	-0.079
17	Permanent swamp, on bank	HPP	-0.131	-0.550
18	Permanent swamp, on vegetation	HPT	-0.068	-0.188
19	Permanent drainage, in plantation, on bank	HPU	-0.320	0.030
20	Permanent drainage, in plantation, in water	HPV	-0.285	-0.007
	Vertical Position			
21	Under surface of soil	VPA	-0.150	-0.040
22	In or under dead leaves	VPB	-0.271	0.022
23	Under rock	VPC	-0.136	0.218

24	Under log	VPD	-0.319	-0.088
25	In log	VPE	-0.253	-0.043
26	On surface of bare soil	VPF	0.542	0.648
27	Surface of leaf litter of dead leaves	VPG	0.196	0.042
28	On rock	VPH	0.085	0.620
29	On log	VPJ	0.002	-0.103
30	On seedling or herbaceous plant (<1 m tall)	VPK	0.599	-0.286
31	On shrub or young seedling (1 - 7 m tall)	VPL	0.622	-0.654
32	On tree or large vine (> 7 m tall)	VPM	-0.102	-0.228
33	On dead stump	VPN	-0.163	-0.231
34	In crown of fallen dead shrub of tree	VPO	-0.103	0.083
35	In grass	VPP	-0.536	0.303
36	On grass blade	VPQ	-0.464	0.013
	Substrate			
37	Leaf of plant	SA	1.617	-0.167
38	Stem or branch of herbaceous plant	SB	0.212	0.214
39	Twig or branch of woody plant	SC	0.033	-0.255
40	Trunk of shrub or tree	SD	0.057	-0.148
41	In epiphyte	SE	-0.280	-0.045
42	Under bark of log, stump, or tree	SF	-0.414	-0.179
43	Bank mud	SG	-0.296	-0.096
44	Bank sand or gravel	SH	-0.228	-0.021
45	Bank rock	SJ	0.100	0.710

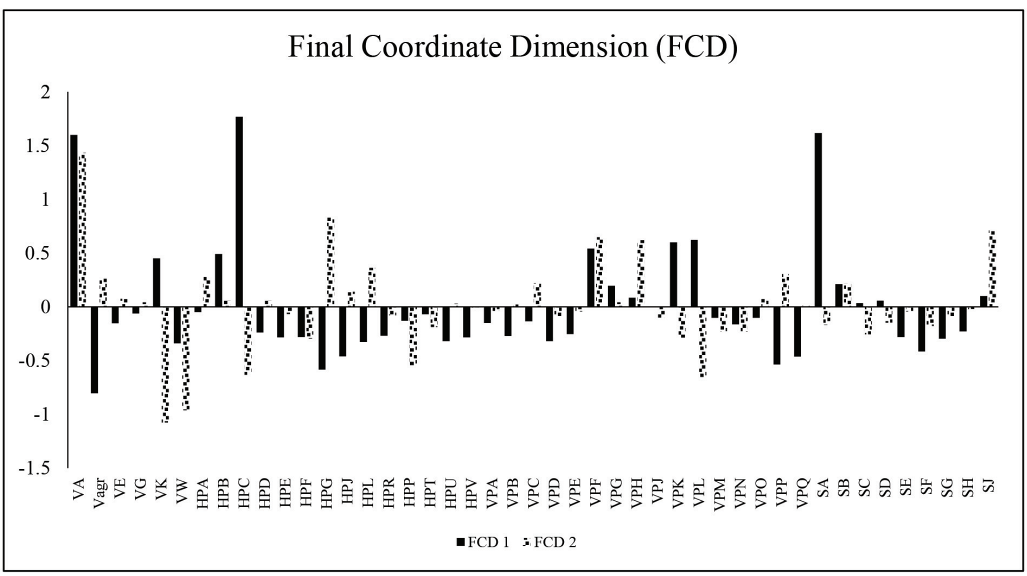

Figure 2: The column chart of final coordinate dimensions, FCD 1 and FCD 2 for each locality generated by NMDS (PROXSCAL)

for Kerangas forest (VK). Additionally, most Sarawak frogs choose to stay on the banks of a permanent stream (HPC) as their preferable horizontal position and this corresponds with the highest positive loading of FDC 1 = 1.768.

Furthermore, the two-dimension values (Table 3 and Figure 2) represent meaningful variables for Sarawak frogs' favourable vertical positions and substrate. Three variables obtained the highest positive loadings, namely the surface of bare soil (VPF) (FCD 2 = 0.648), leaf of plant (SA) (FCD = 1.617) and bank rock (SJ) (FCD 2 = 0.710) with one negative loading of FDC 2 = -0.654 (shrub or seedlings, VPL).

Ecological guilds defined in the scatterplot of the NMDS configuration revealed 7 clusters (Figure 3), namely (i) ground-dwelling frogs of disturbed areas, comprising *Hylarana erythraea*, *Fejervarya limnocharis, Limnonectes paramacrodon* and *Pulchrana baramica*, (ii) arboreal frogs of disturbed areas, comprising *Kurixalus appendiculatus* and *Polypedates leucomystax*, (iii) semi-arboreal (seedlings) frogs of disturbed areas, comprising *Chalcorana raniceps* and *Staurois guttatus*, (iv) ground-dwelling frogs of forest areas, comprising *Kalophrynus meizon, Leptolalax dringi* and *Ingerophrynus divergens*, (v) arboreal frogs of forest areas, comprising *Philautus petersi, Polypedates macrotis* and (vi) semi-arboreal (rock dwellers) frogs of forest areas, comprising *Phrynoides juxtaspera*. The clustering might be due to the adaptability of frog individuals and the availability of food sources. Moreover, some frog species co-exist in the same habitats since they have similar ecological requirements; however, these frog species tend to employ resource partitioning among themselves as can be seen in microhabitat partitioning in the closely related species as described by Zainudin *et al.* (2017).

Examples of microhabitat partitioning in the current study can be seen in other families, such as Megophryidae and Microhylidae, including *Megophrys nasuta* (Mn), *Leptolalax dringi* (Ld) and *Microhyla malang* (Mm), which commonly inhabit leaf litters on the forest floor, under the leaf of seedlings inside the hole of tree log and

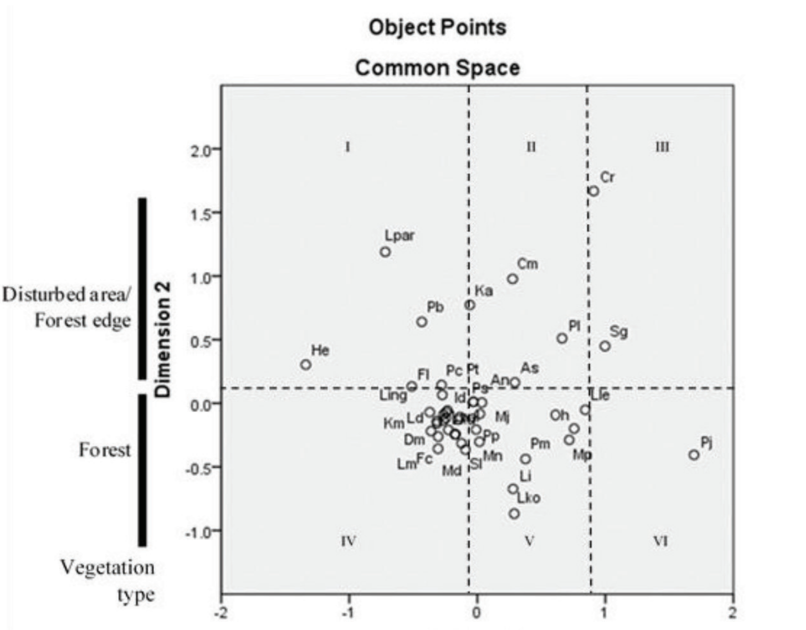

Figure 3: The scatterplot of NMDS configuration showing the ecological guild pattern of each frog species

in the soil. They commonly use leaf litters as their nests and feed on insects that inhibit on the forest floors (Inger *et al.,* 2017). Another example is *Nepenthes ampullaria*, one of the pitcher plant species, which provides shelter and the breeding site for *Microhyla borneensis* (Mb) (Inger *et al.,* 2017). Usually, these forest floor frogs live more than 10 m away from a water source. Colourations also give them the ability to camouflage with the colour of leaf litters on the forest floor. This ability helps ground-dwelling frogs, such as *Megophrys nasuta* (Mn), avoid predators and occupy a good hiding spot beneath the leaf litters (Zainudin *et al.,* 2017 & Zainudin *et al.,* 2019b).

Arboreal frogs that live on trees or higher vertical positions possess morphologies that are designed to live in higher places. They have webbed and dilated toes with broad discs that helps them glide between trees to escape from predators (Inger *et al.,* 2017). *Nyctixalus pictus* (Np), *Philatus tectus* (Pt) and *Philatus petersi* (Pp) are examples of arboreal frogs that perch on seedlings, shrubs and overhanging vegetation. Semi-arboreal frogs such as *Chalcorana raniceps* (Cr) live on overhanging seedlings and shrubs along riverbanks. Sometimes, *C. raniceps* also dwell on the ground and utilise substrates such as dead leaves, dead logs and buttress roots, similar to other forest floor frogs.

However, the ecological clustering scatterplots were influenced by the species or individual microhabitat preferences on each sampling locality. In general, the diversity and distributions of frog species in an area are influenced by the environment, vegetation and landscape (Marques *et al.,* 2018). In this regard, the differences of fauna species depend on their adaptability and survival in a particular habitat. The demand for basic needs such as food sources, shelter and breeding areas are also a priority for species habitat preferences. Therefore, the preservation of the forest area is important to maintain the amphibian's diversity (Gillespie *et al.,* 2012).

The low number of frog species living in open or non-forested areas as compared with forest areas, especially in agricultural or plantation areas is consistent with Gillespie *et al.* (2012) and Zainudin *et al.* (2019a). Only species comensal with humans were able to cope with new surroundings or habitats to ensure the survival of their populations (Marques *et al.,* 2018; Zainudin *et al.,* 2017). This suggests that generalist species such as *Chalorana raniceps* and commensal species such as *Hylarana erythraea* were able to live and adapt to harsh environments (Zainudin *et al.,* 2006). Specialist species, on the other hand, will not be able to survive since they cannot tolerate extreme environmental conditions (Zainudin *et al.,* 2006; Eterovick, 2007; Zainudin *et al.,* 2019a).

The study also found that adult frogs chose their microhabitat preferences, which is consistent with Kopp and Eterovick (2006) and Zainudin *et al.* (2019b). This is because the selection of microhabitats is vital to ensure they are able to forage, as well as the safety of their tadpoles from predators such as snakes (Marques *et al.,* 2018). The existence of amphibian species in various types of forest is influenced by the natural resources and this contributes to the distribution of adult frogs and increases the frogs' diversity in a particular area (Dehling & Dehling, 2021). By sharing the same natural resources, the tendency for two or more frog species to co-exist and assemble in the same niches will also be higher (Afonso & Eterovick, 2007; Zainudin *et al.,* 2017). Since many forests have been cleared for commercial purposes, ecotourism sites, human settlements and development, this has contributed to the decline in frog species (Warguez *et al.,* 2013). Over the past decade, lowland forests were cleared aggressively for timber harvesting and oil palm plantations (Gillespie *et al.,* 2012). Due to continuous anthropogenic effects, amphibians have experienced deterioration in numbers and they were known to be one of the most vulnerable animal groups globally (Gillespie *et al.,* 2012).

Microhabitats are also influential in the selection of a breeding site. Moreover, understanding the importance of microhabitats can help conserve frog communities since the habitats continues to change and forests are actively converted into agriculture areas (Gillespie *et al.,* 2012; Zainudin *et al.,* 2017; Dehling & Dehling, 2021).

Barnett (2005) and Dasi and Shahriza (2020) stated that amphibians need shallow water for breeding, especially warm water for eggs and larvae incubation, with fewer predators from small invertebrates. By applying good safety and preservation of eggs, the adult frog species can sustain the continuity of their tadpoles (Marques *et al.,* 2018). However, sudden events, such as drought and forest burning, can change the environmental factors and reform the microhabitat preference selection, thus increasing the rate of juvenile mortality (Rittenhouse *et al.,* 2008). As frogs are susceptible to any slight change in their surroundings, frog behaviour will mostly depend on the remaining resources available.

Besides, anthropogenic stressors have had a serious impact on frog morphology and behaviour (Marques *et al.,* 2018). As such, the future generations of frogs may experience physical deformities, low immunities and high exposure to disease if their early stages of life are disturbed by anthropogenic stressors (Rittenhouse *et al.,* 2008). Therefore, preservation of the natural landscape needs to be done besides increasing the knowledge of frog species and their microhabitats, which further aids future conservation efforts to ensure that the frog populations can be conserved (Marques *et al.,* 2018; Zainudin *et al.,* 2017).

Conclusion

Frogs exhibit some ecological clustering based on their habitat and microhabitat utilisation. Hence, the identification of ecological guilds is crucial for prioritising conservation. This study can provide prior knowledge of frog species documentation in particular habitats for conservation purposes, ecological data and further research. Future development may lead to habitat disturbance and destruction. Thus, by preserving habitats that are heavily used by frog species as reflected by ecological guilds, it can accelerate a better and successful faunal management that prioritises several areas for conservation purposes. This will lead to a sustainable future for natural resources.

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