Disturbance effects on anuran communities and relative fitness across urban-adjacent forests of Peninsular Malaysia and Malaysian Borneo

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ABSTRACT

Urbanisation profoundly affects anuran biodiversity, particularly species composition and functional traits in natural vegetation contiguous to cities. We assessed anuran community structure and trait variation in two urban-adjacent forests: Ayer Hitam Forest Reserve (AHFR) in Peninsular Malaysia and Universiti Putra Malaysia Bintulu Campus (UPMBC) in Malaysian Borneo, with each system analysed independently within a shared ecological context. We examined biodiversity, abundance, and trait variation across disturbance zones and forest types, while accounting for microclimates and seasonal sampling periods. For AHFR, we integrated published checklist records from 1975 to 1999 with our multi-year monitoring data collected between 2015 and 2019. Standardised nocturnal surveys combined with historical records documented diverse anuran assemblages at both study sites, with disturbance effects most evident in the disturbed zone of AHFR, which showed the lowest rarefied species richness. Anurans in moderately disturbed and disturbed zones of AHFR were associated with variation in body size and condition, with notable responses observed in *Ingerophrynus parvus* and *Kalophrynus palmatissimus*. In UPMBC, planted forest supported relatively resilient species such as *Limnonectes ibanorum*, while species with narrower habitat associations, including *Alcalus baluensis* and *Kalophrynus heterochirus*, showed negative body size deviations. Differences in body size and condition between allopatric populations of *L. ibanorum* in AHFR and UPMBC suggested geographical influences on traits variation. Our study reveals disturbance-driven variation in anurans and conservation relevance.

Subjects Biodiversity, Conservation Biology, Zoology

Keywords Amphibia, Assemblage structure, Body size, Body condition, Modified habitat, Visual encounter surveys, Southeast Asia

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INTRODUCTION

Humans have shaped the landscape for more than 12,000 years (*Ellis et al., 2021*), with a massive impact on species diversity and composition in their natural habitat (*Decena et al., 2020*). Particularly in Southeast Asia, rural and agricultural land development, driven by urban expansion (*Dong et al., 2023*), has been the primary cause of landscape changes. These changes often result in the clear-cutting of forested areas and the canalization of wetlands (*Taufik, Setiawan & Van Lanen, 2019*). These modifications have a profound impact on species, resulting in the documented extinction of megafauna (*Hixon et al., 2022*) and the extirpation of less noticeable groups such as amphibians (*Luedtke et al., 2023*). Amphibians are especially vulnerable to habitat modification because of their permeable skin, life cycles that depending on both terrestrial and aquatic environments, making them sensitive indicators of environmental change (*Nkwonta et al., 2025*). Moreover, urban landscapes represent one of the fastest land-transformation change in Southeast Asia (*Hughes, 2017*), yet their effects on amphibian communities remain poorly understood. Hence, assessing how anthropogenic disturbance shapes anuran community structure and trait variation in urban-adjacent forests is crucial for conservation management in Southeast Asia.

The biodiversity of amphibian fauna in Peninsular Malaysia comprises approximately 107 frog and toad species from six families: Bufonidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae, and Rhacophoridae (*Simon et al., 2022*). In Malaysian Borneo, while 138 species of anurans were known by the end of the last century (*Inger & Lian, 1996*), the number has increased substantially with ongoing taxonomic revisions and new species descriptions, exceeding earlier estimates (*Inger et al., 2017*; *Fukuyama et al., 2021*; *Frost, 2025*), more than 100 species have been recorded in Sabah, northern Borneo (*Kueh & Maryati, 2008*), and Sarawak, western Borneo also hosts a high diversity of anurans, being the focus of continued discoveries and updated inventories (*Matsui, Nishikawa & Eto, 2014*; *Shimada et al., 2015*; *Fukuyama et al., 2021*). Among this rich diversity, species in the family Megophryidae, commonly known as litter frogs, are widespread in Borneo (*Ahmad Sah & Grafe, 2020*), whereas Ranidae represents one of the most diverse anuran families in the region, with complex evolutionary and biogeographic patterns across Sundaland (including Borneo and the Malay Peninsula) and Wallacea, the transitional zone between Asian and Australasian regions (*Reilly et al., 2022*). Yet, how this diversity is organised across forests increasingly shaped by human activities remains poorly understood.

The primary focal site for anurans surveys in this study is the Ayer Hitam Forest Reserve (AHFR), a lowland dipterocarp forest (*Abdul Malek, Majid & Alias, 2005*), adjacent to a highly developed area in Selangor, Peninsular Malaysia (Fig. 1). The forest is inhabited by six identified families of anuran: Bufonidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae, and Rhacophoridae (*Paiman & Yaman, 2007*). However, since AHFR was leased by the local state to a public university in 1996 as a research site (*Mohd Hasmadi, Pakhriazad & Mohamad, 2010*), systematic inventories of anuran diversity were limited prior to 2014 (*Abdul Aziz et al., 2016*). Furthermore, due to its strategic location near the city centre (*Mohd Hasmadi, Pakhriazad & Mohamad, 2010*), AHFR is highly valued but faces

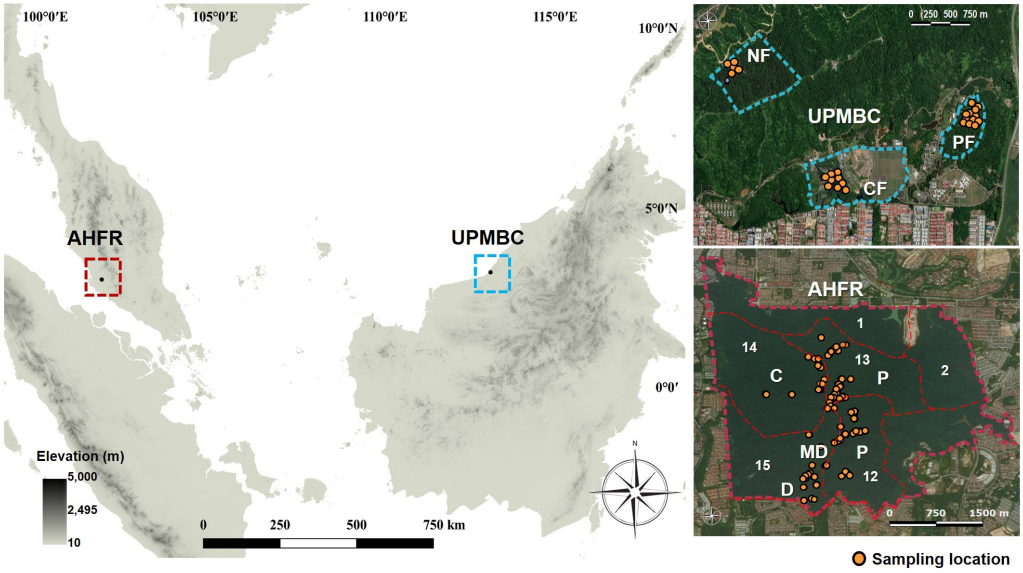

Figure 1 The distribution of species recorded in two focal sites, AHFR in Peninsular Malaysia and UPMBC in Borneo. The numbers shown in AHFR represent the compartments designated by the forest management authority. The abbreviations C, P, MD, and D stand for "controlled", "protected", "moderately disturbed", and "disturbed" zones in AHFR, respectively, based on differences in human access and habitat modification. The abbreviations NF, PF and CF stand for sampling sites "Nirwana forest", "planted forest" and "campus forest" in UPMBC, representing forest types with differing levels of habitat modification and land-use history. Map panels showing sampling locations as points representing survey sites. Detailed species occurrence and sampling information are provided in Table S4 (AHFR) and Table S5 (UPMBC). Basemaps use Stamen Toner Lite (tiles by Stamen Design, CC BY 3.0, Data by OpenStreetMap contributors, ODbL).

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significant threats from ongoing urbanization and surrounding logging activities (*Awang Noor et al., 2007b*).

Similarly, human-induced degradation is evident in the second focal site, a forest near city located in Universiti Putra Malaysia Bintulu Campus (UPMBC; now known as Universiti Putra Malaysia Sarawak, UPMS; Fig. 1), on the west coast of Malaysian Borneo. The UPMBC is one of the few remaining forests near Bintulu, situated approximately 10 km away from the township. Spanning over 60.0 hectares in size, the vegetation landscapes within the UPMBC is structured by fragmented secondary forests that also serves as an educational forest (*Muhd Sahimi, 2017*). The UPMBC rehabilitation forests have been replanted following degradation caused by human activities, such as logging (*Kueh et al., 2011*) and natural vegetation within the campus zone and areas designated for infrastructures (*Ong et al., 2008*). To improve understanding of anuran community structure in forests near cities, it is necessary to document species abundance, diversity and updated checklists, which provide baseline ecological information for evaluating disturbance effects and spatial variation in assemblages.

While habitat use vary widely among anuran taxa and some species have direct development, most anurans rely on aquatic environments for reproduction, including

streams, ponds, and temporary water bodies (*Norhayati, 2017*). Some species also breed in completely shaded microhabitats, including small streams, marsh muds, and under the leaf litter in deep pools (*Matsui, Nishikawa & Eto, 2014*). Consequently, aquatic and riparian habitats play a central role in anuran life cycles. Therefore, conserving water habitats is vital. At the community level, this ecological heterogeneity implies that species detectability and habitat associations vary across taxa and microhabitats. For instance, in Malaysia, many Dicroglossidae species are typically associated with ground-level habitats, near water bodies, particularly during breeding periods (*Norhayati, 2017*). Microhylids like *Gastrophrynoides immaculatus* primarily inhabit leaf litter and bamboo habitat, but also rely on water bodies for breeding activities (*Chan et al., 2009*). Rhacophoridae are mainly arboreal, living in vegetation from shrubs to the forest canopy, but they descend to water bodies for breeding (*Inger et al., 2017*). Many Ranidae species in Malaysia are closely associated with streams, ponds, and other freshwater habitats rather than purely terrestrial environments (*Scott, 2005*). Some species, such as *Limnonectes finchi* and *L. palavanensis*, having partial terrestrial reproductive behaviour, with eggs deposited on land and adult males transported the tadpoles to nearby water bodies (*Vallejos, Grafe & Wells, 2018*). In addition, some Bufonidae species, including stream-associated *Ansonia* spp. and the widespread *Ingerophrynus parvus* are frequently observed in terrestrial habitats near water sources, particularly during rainfall events (*Chan et al., 2014*; *Norhayati, 2017*).

Some anuran species inhabiting primary forests are capable of persisting in secondary forests and other human-modified habitats (*Hilje & Aide, 2012*). Primary forests are generally defined as forests that have not undergone significant anthropogenic disturbance, whereas secondary forests arise following any form of land-use change, such as logging (*Bryan et al., 2013*). Accordingly, certain species may adjust to the disturbed environments and develop ecological or behavioural responses to changes in habitat structure (*Ellepola et al., 2022*), water availability, and food resources (*Camacho-Rozo & Urbina-Cardona, 2021*). For instance, certain species may alter their breeding strategy, or demonstrate increased tolerance towards habitat fragmentation or pollution (*Sievers et al., 2019*), while others may show phenotypic variation, such as changes in call characteristics, in response to anthropogenic environments (*Liu et al., 2022*). In Peninsular Malaysia, several widespread species have been recorded in semi-natural and disturbed habitats, such as *F. limnocharis*, *F. cancrivora*, *Polypedates leucomystax*, *Kaloula pulchra*, *Duttaphrynus melanostictus*, and *Hylarana erythraea* (*Nur Johana et al., 2016*). Similarly, the anthropogenically affected areas within Gaya Island, Sabah, also support diverse anurans, with common species including *H. erythraea*, *F. cancrivora* and *F. limnocharis* (*Sompud et al., 2018*). Given the diverse habitats and species-specific responses to disturbance, combining multiple survey approaches such as transect-based searches, quadrat sampling, and aural surveys provides a more comprehensive assessment of anuran communities than relying on a single method alone (*Borzée, Andersen & Jang, 2018*).

Assessing species diversity, richness, evenness, morphological and conditional traits in natural and human-affected microhabitats can reveal the effects of human pressures on anuran populations and their body condition. Additionally, comparing body size and condition among intraspecific and allopatric populations can provide insights into

species adaptation and guide conservation strategies. To address this, we included two study systems from Peninsular Malaysia and Malaysian Borneo, with analyses conducted independently for each system, to provide a broader ecological context of forests influenced by human activities. Based on our anuran inventories across human-affected and natural areas of AHFR and UPMBC, we hypothesised that more disturbed and modified habitats will show higher species abundance, due to the increasing of generalist or disturbance-tolerance species, but lower diversity, richness, and evenness compared to relatively less disturbed or less modified habitats. This pattern is expected because of habitat and environmental modifications associated with anthropogenic disturbance. We hypothesised that morphological traits such as body size and thigh length, and the physiological trait such as body condition index of anurans, would vary significantly across habitat disturbance gradients and forest types. Specifically, we predicted individuals from more disturbed or modified habitats to have reduced body size and lower body condition, reflecting environmental stress, lack of stable habitats and limited resource availability. We also expected thigh length to vary with habitat structure, as this trait is associated with locomotion and may differ across microhabitats along disturbance gradient. In addition, we expected a pattern of morphological variation among allopatric populations within the same species when comparing individuals from AHFR and UPMBC. Given that the two sites are in distinct longitudinal and environment zones, differences in body size and condition are anticipated to reflect local adaptation or plastic responses to differing habitat quality and ecological pressures. The objectives of the present study were: (1) to characterise anuran community structure including species diversity, richness, evenness and relative abundance in both natural and human-disturbed habitats, with a focus on the two forests nearby cities of AHFR and UPMBC, (2) to assess the effects of human disturbance on four anuran traits: body size, thigh length, body mass, and body condition across forest types in UPMBC and disturbance zones in AHFR, to infer possible adaptive or plastic responses, and (3) to investigate the presence of morphological variation among species occurring in both AHFR and UPMBC, located in Peninsular Malaysia, and Borneo, respectively.

MATERIALS & METHODS

Ethics statement

The study sites include two reserve forests: AHFR in Puchong, West Malaysia (referred to as the Peninsular Malaysia hereafter), and UPMBC in East Malaysia (referred to as Malaysian Borneo hereafter). Both forests are affiliated with Universiti Putra Malaysia (UPM), encompassing the main campus in Serdang and the Bintulu branch campus. Survey permissions were granted through relevant university institutional regulations, including the Faculty of Forestry, Sultan Idris Shah Forestry Education Centre (SISFEC), and Putra Agriculture Centre (UPMBC). Surveys and morphometric measurements were carried out in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines of Universiti Putra Malaysia (approval No: UPM/IACUC/AUP–R007/2018). The approved protocol covered capture, handling, and non-invasive morphological measurements of anurans. No euthanasia was involved, and individuals were released immediately at the

point of capture. All animal handling complied with relevant Malaysian institutional and national wildlife regulations.

Study sites

The AHFR is located three to six kilometres from the closest cities, Kinrara and Puteri, in Puchong, Selangor state of Peninsular Malaysia. This secondary forest includes four types of landscapes: natural and ornamental vegetation, crops and farmland, grassland, and artificial built-up areas (*Marina et al., 2021*). As a secondary lowland dipterocarp forest, AHFR has experienced substantial anthropogenic disturbance, including logging, land conversion, and infrastructure development. Historical records indicate extensive forest loss over a relatively short period in the late 20th century (*Awang Noor et al., 2007a*), contributing to pronounced spatial heterogeneity in disturbance intensity across the forest reserve. Between 2004 to 2019, we conducted surveys across AHFR, encompassing four zones characterised by differences in location, size, and levels of anthropogenic disturbance. While disturbance was not quantified using continuous variables, the classification is grounded in documented long-term anthropogenic modification of AHFR (*Awang Noor et al., 2007a*; *Awang Noor et al., 2007b*) and reflects consistent differences in human access, infrastructure, and habitat alteration across zones, including recreational use by surrounding communities (*Mohd, Yaman & Jamaludin, 1999*) and variation in road proximity and habitat modification defining disturbance gradients in the reserve (*Sanei & Zakaria, 2011*). These zones are described as follows: (1) a protected zone, located at the centre of AHFR (3.94°N, 100.88°E) and at the southeastern range of AHFR (3.22°N, 100.861°E), characterised by secondary forests that were previously logged and underwent rehabilitation efforts between 1936 and 1951 to improve forest resources (*Paiman & Yaman, 2007*); (2) A controlled zone (low to moderate disturbance), located at the northern range of AHFR (3.02°N, 101.639°E), characterised by secondary forest with human disturbance as joggers and hikers from adjacent settlements who frequently access this zone. However, access was regulated for selective activities aimed at increasing forest coverage; (3) A moderately disturbed zone located at the southwestern range of AHFR (3.00°N, 101.610°E), characterised by secondary forests that have both areas with frequent human presence, and the areas consist of mostly natural vegetation, replanted trees, forest litter, walking trails, streams, swampy areas, rivers and sandbank and drainage area; (4) A disturbed zone, located at the southern range of AHFR (2.99°N, 101.644°E), characterised by disturbed secondary forest due to the development of man-made buildings such as post guard, nursery, basecamp, chalet, bus stop, administration facilities and laboratories. This zone is also divided by a main road used by vehicles to enter the forest reserve, and the landscape in this zone includes walking trails, and man-made buildings, such as a demonstration area, administrative office, post guard, nursery, basecamp and laboratories for demonstration (Fig. 1). To complement the surveyed zones in AHFR, historical checklist data from a minimally disturbed area were consulted as contextual information only. The data originated from inventory checklists conducted between 1975 and 1999 along the Rasau and Bohol rivers in the central to eastern parts of AHFR (*Haji Ali, Rajagopal & Yaacob, 1999*). This minimally disturbed category

was excluded from all quantitative statistical analyses to avoid bias arising from differences in sampling methodology and survey effort.

Universiti Putra Malaysia Bintulu Campus (UPMBC), located 13 kilometres (km) from the Bintulu township (3.2086°N, 113.0978°E; Fig. 1), is a secondary forest that was logged in the 1980s and then abandoned without management (*Jamaluddin et al., 2013*). In contrast to AHFR, analyses in UPMBC were conducted independently using forest type as the primary grouping factor. The anurans survey focused on the three sampling sites in UPMBC, characterised by forest type based on vegetation structure and land-use history rather than a disturbance gradient: (a) Nirwana forest, one of the natural secondary forests located at the northwestern part of UPMBC (*Muhd Sahimi, 2017*), (b) planted forest, a rehabilitated forest that preserved after degradation by logging activities (*Kueh et al., 2011*), located at the eastern range of UPMBC, and (c) campus forest, composed of natural vegetation within the campus zone and located in the central part of UPMBC (Fig. 1).

Nocturnal visual encounter surveys across habitats and zones

Biodiversity survey in AHFR and UPMBC followed a comparable framework based on nocturnal visual encounter surveys, random walking transects and fixed-area quadrats where linear transects were impractical. Minor site-specific adjustments accommodated differences in terrain, microhabitat structure, accessibility and safety conditions in Peninsular Malaysia and Malaysia Borneo, without altering the fundamental survey design, enabling cross-regional comparison of anuran diversity and trait variation.

In AHFR, we conducted survey between 2015 and 2019. Sampling was carried out within defined temporal windows, with most surveys in protected, controlled and moderately disturbed zones conducted between October and February, corresponding to periods of relatively higher rainfall. While surveys in the disturbed zone were conducted between March and July, representing relatively lower rainfall periods, largely due to site accessibility and proximity to human settlements. All survey activities were consistently conducted every Friday and Saturday night, twice a month, for three and half hours, from 2030 to 0000. In tropical Malaysia, rainfall occurs throughout the year, therefore, the terms ‘‘wet’’ and ‘‘dry’’ seasons in this study refer to relatively wetter, and less-wet sampling periods rather than discrete climatological seasons. These terms were strictly used for analytical convenience in statistical modelling and do not imply strict climatic seasonality. Because most sampling in the disturbed zone occurred during relatively less-wet periods, potential seasonal influences on survey outcomes were explicitly accounted for in subsequent analyses. Microclimate data were collected *in situ* across the protected, controlled, and disturbed zones as part of ongoing biodiversity surveys (Table S1), including temperature, humidity, wind, light intensity, soil pH, and soil moisture. These measurements reflect environmental conditions experienced by amphibians at encounter locations, rather than a controlled experimental design. Rainfall was not recorded at the sampling-unit level and was therefore treated as contextual information rather than a predictor variable. Potential seasonal influences associated with precipitation were accounted for by including sampling season in subsequent analyses, while humidity and soil moisture provided direct proxies of local habitat conditions. Preliminary analyses detected no statistically significant

microclimatic differences among zones (ANOVA and Kruskal–Wallis tests showed $P > 0.05$ for all variables; Table S2). This comparison was conducted within AHFR only, consistent with the independent analysis of the AHFR and UPMBC study systems. Although no significant seasonal variation in microclimate variables detected across zones, we retained season of sampling as a fixed effect in all models due to its ecological relevance and potential to confound spatial patterns. To further account for temporal variability, we included year of sampling, as the dataset spans multiple years, including historical samples, during which climatic conditions and disturbance regimes may have fluctuated.

In each sampling zone, we established transect lines of approximately 300 m with sampling points spaced at 15 m intervals. Transects were positioned primarily along streams, walking trails, and accessible disturbed habitats where linear surveys were feasible. In swampy areas where transect placement was impractical, we used fixed-area quadrats (5 $\times$ 5 m, 25 m^2 each) as complementary sampling units. Transect length occasionally varied due to terrain constraints, and the number of transects therefore differed among zones according to habitat extent and local environmental conditions. Overall, we established 36 transect lines across AHFR, including six in the controlled zone, 18 across the protected and moderately disturbed zones, and 12 in the disturbed zone. In swampy habitats of the protected and moderately disturbed zones, nine quadrats (5 $\times$ 5 m, total sampled area 225 m^2) were established as independent sampling units, spaced at least 100 m apart to reduce spatial autocorrelation, and distanced 10 m from streams to minimise overlap with transect-based sampling. In the disturbed zone, where natural trails were limited, we established 12 transect lines in built-up and partially vegetated settings, including areas near administrative buildings, reserve facilities, and laboratory surroundings.

UPMBC surveys were conducted intermittently between 2005 and 2009. Surveys in the Nirwana, campus and planted forests were conducted between December and March, spanning months with relatively higher rainfall at the beginning of the period and moderate precipitation thereafter. As in AHFR, these temporal categories represent relative precipitation conditions in a tropical context rather than discrete seasonal divisions. Because sampling effort was structured primarily by forest type, finer time-based separation was not feasible, and temporal variation was incorporated as a categorical factor in the linear mixed-effects models (LMMs).

Anuran surveys across AHFR and UPMBC employed nocturnal visual encounter surveys using random walking, transects, or quadrat-based sampling depending on habitat configuration. Survey effort was standardised across sampling units through consistent survey duration, timing, and observer teams to minimise detection bias and ensure comparability among sites despite differences in accessibility, habitat configuration, and environmental conditions. Individuals encountered were temporarily handled for species identification, morphometric measurements, and photographic documentation before release at the capture location. Detailed survey protocols, morphometric procedures, and species identification criteria are provided in the Supplementary Materials, with a summary comparison of survey design between the two study sites (Table S3).

Statistical analyses for community structure

We calculated species richness, Shannon diversity index (H′), and Pielou's evenness index (J′) to characterise anuran community structure across disturbance zones in AHFR and forest types in UPMBC. We calculated Shannon diversity as:

$$H^{'} = -\sum_{i=1}^{s} p_i \ln p_i$$

where (H') represents Shannon Diversity Index, (S) represents the total number of species in the community (species richness) in the targeted range, and (p_i) is the proportion of individuals belonging to respective species out of the total number of anurans individuals. We assessed community composition by analysing species richness and Pielou's evenness index (J′) across sampling units in AHFR and UPMBC. We defined species richness as the total number of species recorded per sampling unit and calculated evenness as:

$$J = \frac{H'}{\log(S)}$$

where H' represents the Shannon entropy derived from the species abundance distribution, and S is species richness.

We avoided direct comparisons of Shannon diversity (H′) across sampling zones and forests in AHFR and UPMBC due to potential biases arising from differences in sampling methods, effort, duration, species richness, and evenness (see Tables S4–S5). Instead, we compared alpha diversity using individual-based rarefaction to standardise species richness across sampling units with unequal sampling effort and fitted rarefaction curves to species richness matrices (*Willis, 2019*). To standardise species richness across sampling units with varying effort, we applied individual-based rarefaction. As sampling years in UPMBC were not continuous (no data from 2006–2007), and methods of survey varied slightly (random walks across quadrat *vs.* stream *vs.* trail), we standardised effort to reduce detection bias and account for uneven replication. We defined the sampling units for UPMBC based on combinations of forest type (planted forest, campus forest and Nirwana forest), sampling method (random walks across quadrat, streams and trails), and survey duration, ranged from 157.5 to 273 h (Tables S6–S7). For the AHFR dataset, we defined our sampling units based on zone, survey method (line transects across streams, trails, swamps, built-up, vegetated area and quadrat plots), and duration of sampling within each zone, ranged from 312 to 612 h (Tables S8–S9). For each sampling unit in AHFR and UPMBC, we constructed species abundance matrices and generated rarefaction curves in R using the vegan package (*Oksanen, 2010*). To improve the accuracy and comparability of species richness estimates, we have revised the cutoff to reflect the minimum number of individuals sampled across the respective forest types (UPMBC) and disturbance zones (AHFR).

To complement species richness based on rarefaction, we assessed the trends of population abundance using Catch Per Unit Effort (CPUE). The CPUE standardises species abundance relative to effort, providing insight into how common or dominant species are in different habitats or conditions (*Allen, Roberts & Bauder, 2020*). For standardisation, we calculated as the number of individuals observed per 1,000 sampling hour for both AHFR

and UPMBC. This analysis providing abundance index of the species sampled for each site, adjusted with effort of sampling, using the formula:

$$CPUE = \left(\frac{number\ of\ individuals}{survey\ hours} \right) \times 1000.$$

Using the standardisation, we compared relative species abundance and population trends over time across habitat types and disturbance levels. We identified five most abundant anuran species based on their total CPUE values summed across all sampling units.

The analytical approaches for species evenness, rarefaction and CPUE followed the same methodology, with adjusted grouping factors for AHFR and UPMBC. We conducted all of the statistical analyses for species richness, evenness and rarefaction in the R statistical environment using the Vegan and Fossil Packages, the calculations of CPUE and data visualisations were conducted using dplyr (*Wickham et al., 2018*) and ggplot2 (*Wickham, 2011*), with RStudio and R v.4.2.3 (*R Core Team, 2024*).

Linear mixed-effect models across full datasets

We used linear mixed-effect models (LMMs) to account for the hierarchical structure of the morphological dataset and the non-independence of observations within species. We analysed morphological data of anurans collected across four disturbance zones in AHFR, and three forest types in UPMBC (campus, planted, and Nirwana forests), to evaluate the effects of human disturbance and habitat modification on body size and limb morphology across taxa in the community. To account for allometric scaling on morphological traits, we adjusted thigh length (TL) and body mass (where available) against snout-vent length (SVL) using residuals from linear regressions on TL and body mass on SVL. We estimated body condition index (BCI) based on data availability. For AHFR, where body mass was available, BCI was calculated as residuals from a regression of body mass on SVL, which provides a size-adjusted index of individual condition (*Peig & Green, 2009*). For UPMBC, body mass measurements were not available due to logistical constraints during field sampling, body condition was estimated using a morphology-based proxy of body condition, derived using a linear model incorporating SVL and size-adjusted TL, following *MacCracken & Stebbings (2012)* using the following linear equation:

$$Trait = \beta_0 + \beta_1 \times SVL + \beta_2 \times TL + \varepsilon$$

where, β_0 indicates the intercept, β_1 and β_2 are the coefficients for SVL and TL, respectively, and ε is the error term. We conducted all of the statistical analyses using Packages Vegan in RStudio and R v.4.2.3 (*R Core Team, 2024*).

Because morphometric coverage varied among forest types in UPMBC, SVL was analysed across all forest types ($n = 94$; Table S5), whereas TL and BCI were analysed for individuals with complete measurements only ($n = 41$; Table S6). For UPMBC, LMMs included forest type and microhabitat as fixed effects, with species fitted as a random intercept to account for interspecific morphological variation. We assessed model assumptions using Q-Q plots and residuals diagnostics. In addition, we conducted Kruskal–Wallis tests followed by

Bonferroni-adjusted Dunn's *post hoc* comparisons to evaluate differences among forest types, microhabitats, and species under non-parametric conditions.

For AHFR, we initially analysed the full dataset included 33 anuran species across all disturbance zones, comprising 261 individuals with complete morphological measurements (SVL, TL and body mass: Table S8). We fitted LMMs with SVL, TL, body mass and BCI as response variables, disturbance zone as a fixed effect, and species as a random intercept to account for interspecific variation. We also modelled microclimatic variables (soil pH, humidity, temperature), sampling season, and sampling year as model covariates, to account for environmental and temporal variation. These environmental variables were explicitly incorporated to account for local habitat conditions and to improve interpretation of ecological and morphological variation across disturbance gradients. Normality of residuals was assessed using Q-Q plots and residual diagnostics. Body mass however, deviated from normality, therefore we applied Box–Cox transformation to body mass and BCI for LMM analyses. For non-parametric comparisons using Kruskal–Wallis tests with Bonferroni-adjusted Dunn's *post hoc* comparisons based on untransformed body mass data and log-transformed BCI. Further details on AHFR model selection and sensitivity analyses are provided in the Supplementary Methods.

For both the UPMBC and AHFR datasets, we estimated marginal means and performed pairwise contrasts for the responsive factors of body size and condition variables using the Emmeans Package (*Lenth, 2025*). We computed the model fitting and significant testing using lme4 and lmerTest Packages (*Kuznetsova, Brockhoff & Christensen, 2017*), followed by the Kruskal–Wallis and *post-hoc* tests using FSA Package. All analyses were conducted in RStudio and R v.4.2.3 (*R Core Team, 2024*).

Variance in body size and condition traits across targeted datasets

Because the AHFR dataset provides more comprehensive morphometric information than UPMBC, we first examined variation in body size and BCI across disturbance zones using the full AHFR dataset of 33 anuran species. LMM were used to assess zone-level differences while accounting for interspecific variation by modelling species as a random intercept. We then conducted a finer intraspecific analysis focusing on five widespread species with balanced representation across zones to evaluate how disturbance influences SVL, TL and body condition at the species level. Reliable sex determination was not possible during our nocturnal surveys, as many anuran species lack consistent external sexual characters and calling males often cease vocalising when approached, a common challenge in tropical forests (*Köhler et al., 2017*). Consequently, we modelled species as a random effect in the LMM using the formula: Trait∼Zone + (1|Species), implemented in the lme4 package in R v.4.2.3 (*R Core Team, 2024*). This specification allows each species to have its own mean and variance and absorbs unmeasured intraspecific variation, including potential sex-related differences. Such treatment of species as a random intercept is standard in ecological trait analyses when sex cannot be included explicitly, because any sex differences that do exist are nested within species and therefore, captured as random variation associated with species identity (*Harrison et al., 2018*).

Based on the full AHFR LMM outputs, we identified seven species that showed significant zone-level trait responses (df individual = 99; Fig. S6). To ensure adequate within-species representation, we excluded species with extremely low sample sizes (*Pulchrana baramica*, $N = 4$ and *Occidozyga laevis*, $N = 6$), and retained only species with ≥15 individuals for further non-parametric testing using Kruskal–Wallis analyses followed by Dunn's *post hoc* comparisons. The resulting focal species were: *Pulchrana laterimaculata* (N individual = 19: disturbed *vs.* controlled *vs.* protected), *Leptobrachium ingeri* (N individual = 17: controlled *vs.* disturbed *vs.* moderately disturbed *vs.* protected), *Ingerophrynus parvus* (N individuals = 21: controlled *vs.* disturbed *vs.* protected), *Kalophrynus palmatissimus* (N individual = 15: controlled *vs.* disturbed *vs.* moderately disturbed *vs.* protected) and *Limnonectes ibanorum* (N individual = 17: moderately disturbed *vs.* protected). In addition, we included traits measured from seven species that were widespread in AHFR, distributed across at least three disturbance zones: *Microhyla bermorei*, *M. mantheyi*, *Fejervarya limnocharis*, *F. moodiei*, *P. laterimaculata*, *I. parvus*, and *L. ingeri* as an alternative targeted subset. Although these species did not consistently exhibit significant variation in the LMMs, they were retained for comparative purposes, with their results presented in the supplementary materials for reference (Fig. S6; see dataset in Table S9).

AHFR and UPMBC are geographically and longitudinally disconnected forests (Fig. 1). Despite this, *Limnonectes ibanorum* occurs in both sites, and was selected for species-specific modelling for morphological comparisons due to its abundant and reliable data replicates. For the statistical analyses, we repeated the same normality test procedure as in the previous datasets. We conducted a Principal Component Analysis (PCA) biplot to understand the clustering pattern of variables that contribute most significantly to the observed variation. We calculated eigenvalues, which are equivalent to variance and standard deviation squared. The PCA biplot identifies the clustering of variables contributing to observed variation, while the Mann–Whitney-Wilcoxon test assesses differences between intraspecific groups when assumptions of normality and homogeneity of variance are not met. Since TL did not meet these assumptions, we applied the test to determine if there was a significant difference between the two intraspecific groups of *L. ibanorum*. We performed all statistical analyses using 'MASS' (*Venables & Ripley, 1997*), 'car' (*Fox & Weisberg, 2019*), 'FSA' (*Ogle et al., 2023*), 'Factoextra' (*Kassambara & Mundt, 2020*), and ggplot2 (*Wickham, 2011*) Packages, within the R statistical environment using RStudio and R v. 4.2.3 (*R Core Team, 2024*).

RESULTS

Anuran community structure across study sites

Anuran communities differed across disturbance gradients in AHFR and among forest types in UPMBC. In addition, CPUE estimates indicated species-specific responses to habitat conditions, although detectability differences suggest these values reflect relative rather than absolute abundance (Fig. 2). In AHFR, the disturbed zone supported the highest abundance (191 individuals; 40.98% of records) but showed lower diversity (H' = 2.12) and evenness ($J = 0.72$), indicating dominance by certain taxa (see detailed community inventories in Tables S8–S14). Rarefaction analyses similarly suggested lower standardised

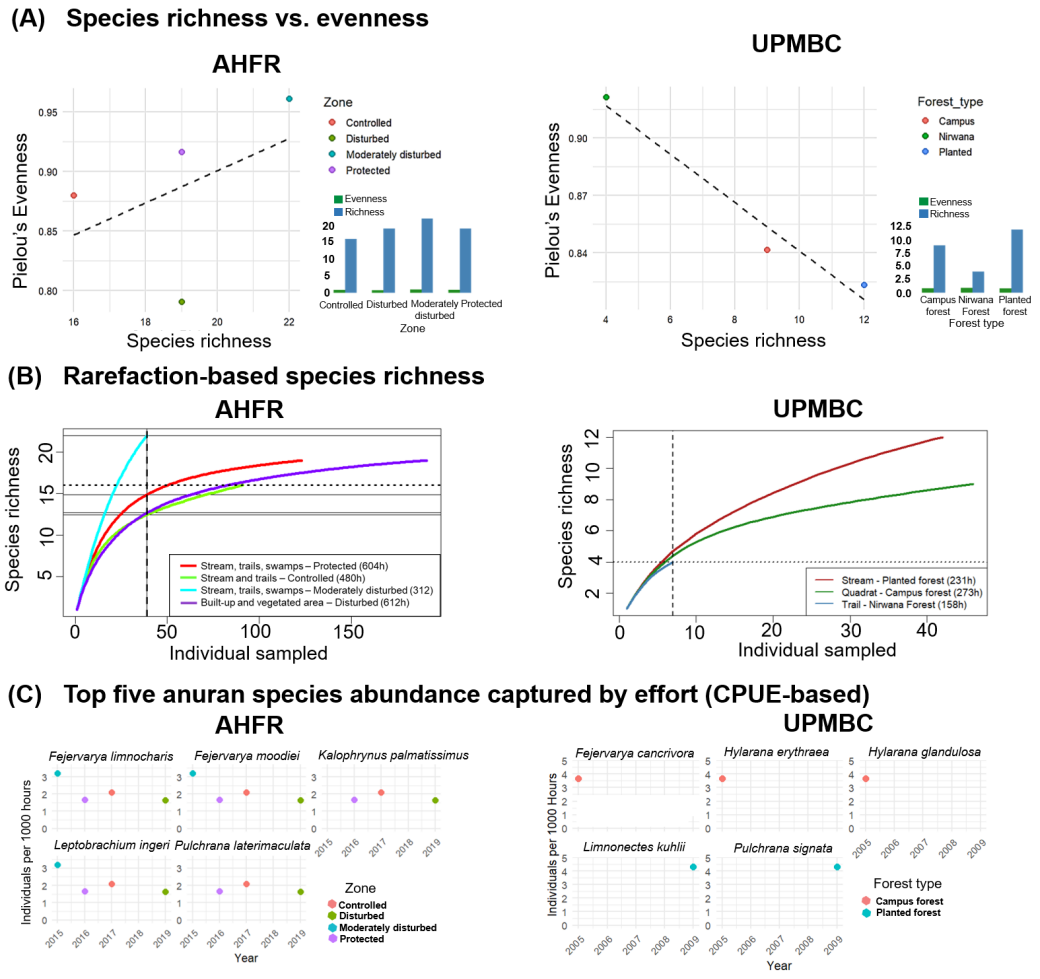

Figure 2 Patterns of anuran species diversity and relative abundance across two study sites in Malaysia. The study sites are forests near city, Ayer Hitam Forest Reserve (AHFR) and Universiti Putra Malaysia Bintulu Campus (UPMBC). (A) Relationship between species richness and Pielou's evenness across disturbance zones (AHFR) and forest types (UPMBC). Insets show corresponding richness and evenness values. (B) Rarefaction-based species richness curves accounting for variation in sampling effort (in hours) and number of individuals sampled, stratified by zone (AHFR) and forest type (UPMBC). Dashed lines indicate curves represent the estimated maximum species richness expected if sampling effort were increased indefinitely. (C) Temporal trends in effort-standardised abundance for CPUE (individuals per 1,000 h) of the five most frequently recorded anuran species per site. Points represent annual abundance values colored by zone (AHFR) or forest type (UPMBC).

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richness in this habitat compared with protected zones (18 species at comparable effort; Fig. 2, Fig. S1). In UPMBC, the planted forest showed the highest richness (12 species) but lower evenness (0.86), whereas Nirwana forest showed moderate richness (10 species) with higher evenness (0.90). The campus forest had lower richness overall (8 species; evenness 0.88; see detailed community inventories in Tables S15–S16; Fig. S2).

Impact of forest type and microhabitats on anurans body size and condition

Linear mixed-effects models revealed a significant effect of forest type on SVL in anurans sampled from UPMBC (F (2, 22.45) = 23.54, $P < 0.001$), indicating substantial variation in body size among forest types (Fig. 3A). Estimated marginal means further supported this pattern, showing that anuran SVL in the planted forest was greater (122.2 mm) than those in the campus forest (41.7 mm) and Nirwana forest (55.9 mm; Fig. S3). Pairwise contrasts indicated that SVL did not differ significantly between anurans in campus (–14.2 mm, $P = 0.571$) and Nirwana forest, while anurans in the planted forest remained significantly larger than those in both other forest types ($P < 0.001$; Fig. S3). Pairwise comparisons from Dunn's test (Bonferroni-corrected) supported the LMM findings, showing significantly lower SVL in anurans from planted forest compared to campus forest (Z = −8.27, P adjusted < 0.001).

LMMs showed no significant effect of microhabitat type on SVL (F(9, 20.86) = 1.94, $P = 0.102$; Fig. S3), consistent with species-adjusted patterns observed across microhabitats (Fig. 3B). However, non-parametric analyses, the Kruskal–Wallis test and *post hoc* Dunn's comparisons detected significant differences among certain microhabitats, particularly for individuals associated with under rocks (Z = −5.31, P adjusted = 4.98×10^{-6}), riverbanks ($Z = 4.75$, P adjusted = 9.10×10^{-5}), open field ($Z = 3.57$, P adjusted = 0.016), and riverbanks combined with open field ($Z = 5.16$, P adjusted = 1.09×10^{-5}). SVL of individuals inhabiting moist shaded areas were significantly lower than those in riverbanks and open field (Z = −3.77, P adjusted = 0.0072). Our results showed that microhabitat influences on SVL may occur locally but are less consistent when accounting for species-level variation in the LMM framework.

While broader environmental conditions associated with forest type are likely the primary drivers of body size variation in UPMBC, localized habitat features influence SVL to some extent, particularly for tree-dwelling individuals. Large-bodied species such as *L. ibanorum*, *Polypedates otilophus*, and *Megophrys nasuta* showed strong positive deviations from the mean and were predominantly found in the planted forest, which is moderately disturbed and structurally open (Fig. 3C). Similarly, *Phrynoidis juxtasper* and *Pulchrana picturata* were largely associated with this habitat. In contrast, smaller species like *Alcalus baluensis*, *Limnonectes paramacrodon,* and *P. baramica* showed negative deviations and were linked to shaded or less disturbed areas particularly within Nirwana forest (Fig. 3A), and in microhabitats such as riverbanks (Fig. 3B). We note, however, that increased visibility in more open habitats may enhance detection of larger-bodied species, therefore, the relative contributions of ecological processes and detectability cannot be fully separated with the present data.

Additionally, variation in body size and condition among anurans in the planted forest was primarily attributed to species differences (random effect variance = 5.15; Fig. 3C), rather than microhabitat type (Fig. 3B). Neither TL nor BCI differed significantly across microhabitats (F(5, 2) = 0.082, $P = 0.988$; Kruskal–Wallis: $\chi^2 = 0.90$, $P = 0.970$), although slightly higher TL and BCI values were observed in individuals inhabiting waterbodies (Emmeans = 1.31, SE = 2.35, $P = 0.586$; Fig. 3D). However, within the planted forest,

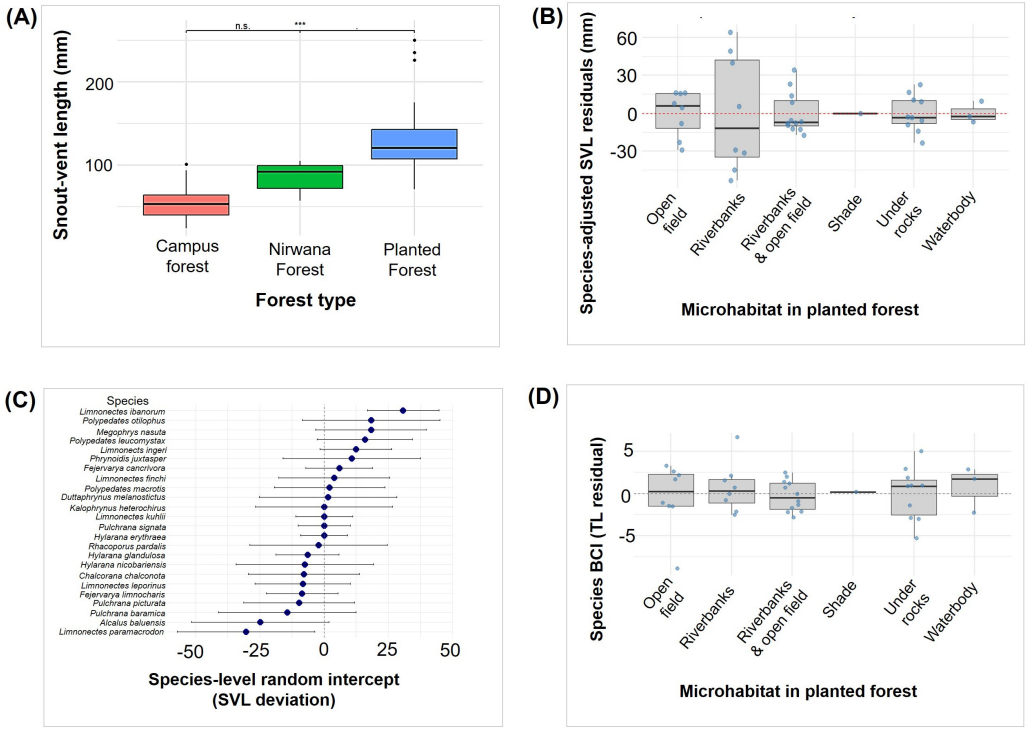

Figure 3 Effects of forest type, microhabitat, and species identity on anuran body size and condition in UPMBC. (A) Snout-vent length (SVL) across forest types in UPMBC. Boxplots show that anuran body size differed significantly among forest types, with individuals from planted forest exhibiting significantly larger SVL than those from Nirwana and campus forests ($P < 0.001$), while no significant difference was detected between Nirwana and campus forests. (B) Species-adjusted SVL residuals across microhabitats within the planted forest in UPMBC. Boxplots represent within-community variation in body size after accounting for species identity, illustrating modest differences in SVL distributions among microhabitats. (C) Species-level random intercepts from the linear mixed-effects model, showing interspecific variation in SVL. Larger-bodied taxa (*Limnonectes ibanorum*, *Polypedates otilophus*) exhibited positive deviations from the overall mean, whereas smaller-bodied species (*e.g.*, Alcalus baluensis, Limnonectes paramacrodon) showed negative deviations. (D) Species-adjusted body condition index (BCI), calculated as residual tibia length (TL) after correcting for SVL, across microhabitats within the planted forest in UPMBC. No significant differences in BCI were detected among microhabitat types (Kruskal–Wallis test), and no consistent directional patterns were observed.

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Kruskal–Wallis tests revealed significant differences among microhabitats($\chi^2 = 17.21$, $P = 0.004$), particularly between individuals from riverbanks and those from riverbanks combined with open field habitats ($Z = 3.22$, P adjusted $= 0.019$: Fig. 3C), suggesting that fine-scale habitat structure may influence body size of anurans under specific conditions.

Intraspecific variations in body size and condition across disturbance zones

In AHFR, the LMM indicated that humidity alone did not significantly explain variation in anuran body size. Notably, both the protected and controlled zones supported larger individuals despite having lower humidity levels. In contrast, the disturbed and controlled zones experienced similarly high humidity, yet anurans from these zones having different

body sizes. Seasonal sampling also did not align with body size patterns, as anurans in the controlled zone (wet season) were larger than those in the disturbed zone (less wet season), despite the expected association between higher humidity and larger body size (Fig. 4). Kruskal–Wallis and *post hoc* Dunn's tests indicated that habitat disturbance in AHFR influenced SVL, body mass and BCI across several anuran species (Figs. 4 and 5).

Estimated marginal means revealed differences in community-level SVL among disturbance zones in AHFR, with smaller average body sizes observed in more disturbed habitats. Anurans in the controlled zone showed larger body sizes compared to those from moderately disturbed (pairwise contrast = 14.9 mm, $P < 0.001$) and disturbed zones (pairwise contrast = 7.92 mm, $P = 0.049$). Individuals from the moderately disturbed zone were smaller than those from the protected zone (pairwise contrast = –12.8 mm, $P < 0.001$). These patterns support the association between habitat disturbance and body size at community level (Fig. 4), although such differences are not consistently observed within species. However, species-specific responses were limited for both SVL and body mass, with significant SVL variation detected only in *I. parvus* (Fig. 5).

Body Condition Index also varied among zones, with anurans in disturbed zones showing higher BCI ($P < 0.001$), and lower BCI in the moderately disturbed zone ($P = 0.003$). This pattern was consistent with the non-parametric analysis, which showed significant differences in BCI among zones ($\chi^2 = 44.252$, $P < 0.001$; Table S19), with the disturbed zone differing from the other habitat types (Dunn's test, $P < 0.05$; Table S19 Fig. 5). Species-level analyses indicated that zone-related differences in BCI were detectable in several focal taxa, including *Ingerophrynus parvus*, *Kalophrynus palmatissimus*, *Leptobrachium ingeri*, and *Limnonectes ibanorum* (Table S19; Fig. 5), although the magnitude and direction of these differences varied among species.

Thigh length did not vary significantly across zones or seasons in AHFR, with Kruskal–Wallis tests and estimated marginal means showing overlapping confidence intervals and non-significant pairwise contrasts (Fig. 4). Species-specific analyses further revealed no significant differences in thigh length among forest zones for any of the focal species (Dunn's test, P.adj >0.05; Fig. 5). Overall, because morphometric traits are influenced by ontogeny and species-specific growth trajectories, these patterns are interpreted cautiously as population-level summaries rather than direct responses to abiotic or biotic conditions.

Body size and condition among allopatric populations in Peninsular Malaysia and Borneo

The PCA biplot provides insight into the potential functional ecology of species by linking their morphological traits such as SVL, TL and BCI with their ecological strategies in AHFR and UPMBC (Fig. 6). In AHFR, the PCA biplot showed that *Fejervarya limnocharis* had a positive correlation with TL, while the other species, *Pulchrana laterimaculata*, *Leptobrachium ingeri*, *Ingerophrynus parvus*, *F. cancrivora*, and *Microhyla mantheyi* showed a stronger correlation with SVL (Fig. 6). This suggested that *F. limnocharis* may be more adapted to environments that require greater mobility or aquatic adaptation. Conversely, among the species representatives observed in UPMBC, *Limnonectes ibanorum* showed a correlation with SVL, whereas the others were correlated with TL (Fig. 5). This suggested

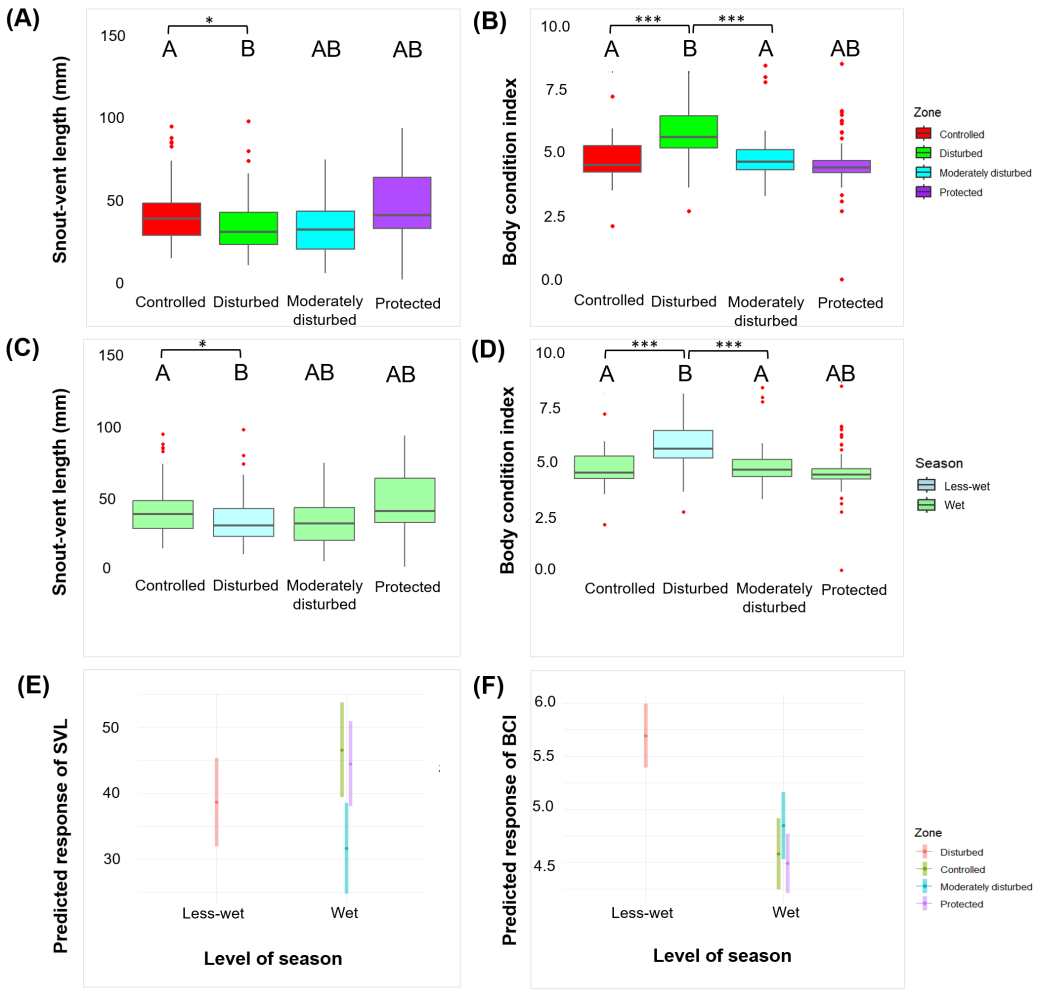

Figure 4 Predicted variation in snout-vent length (SVL) and body condition index (BCI) of anurans across disturbance zones and seasonal sampling periods in AHFR, based on linear mixed model (LMM) analyses. (A–B) represent estimated marginal means and 95% confidence intervals for SVL (left) and BCI (right) across four disturbance zones (controlled, disturbed, moderately disturbed, and protected). (C–D) represent zone-level comparisons of SVL (left) and BCI (right) separated by seasonal sampling periods (wet *vs.* less-wet), highlighting interaction patterns between habitat disturbance and seasonal conditions. Panels E–F represent model-predicted responses for SVL (left) and BCI (right) by season, with coloured bars representing different disturbance zones. Here, "wet" and "less-wet" refer to seasonal sampling periods rather than direct measures of precipitation, as clarified in the main text. Letter groupings (A, B, AB) above boxplots indicate significant differences among zones based on *post hoc* comparisons; groups sharing the same letter are not significantly different, whereas the absence of letter labels indicates no significant variation. Asterisks denote levels of statistical significance ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). All LMMs included species as a random intercept to account for interspecific variation. Significance levels are based on *post hoc* pairwise contrasts using Kenward-Roger degrees of freedom and Tukey-adjusted *P*-values.

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L. ibanorum may be more adapted to behaviors such as territoriality or foraging strategies that align with the habitat in UPMBC. The PCA biplot revealed intraspecific variation between *L. ibanorum* populations from AHFR, Peninsular Malaysia, and UPMBC, Borneo,

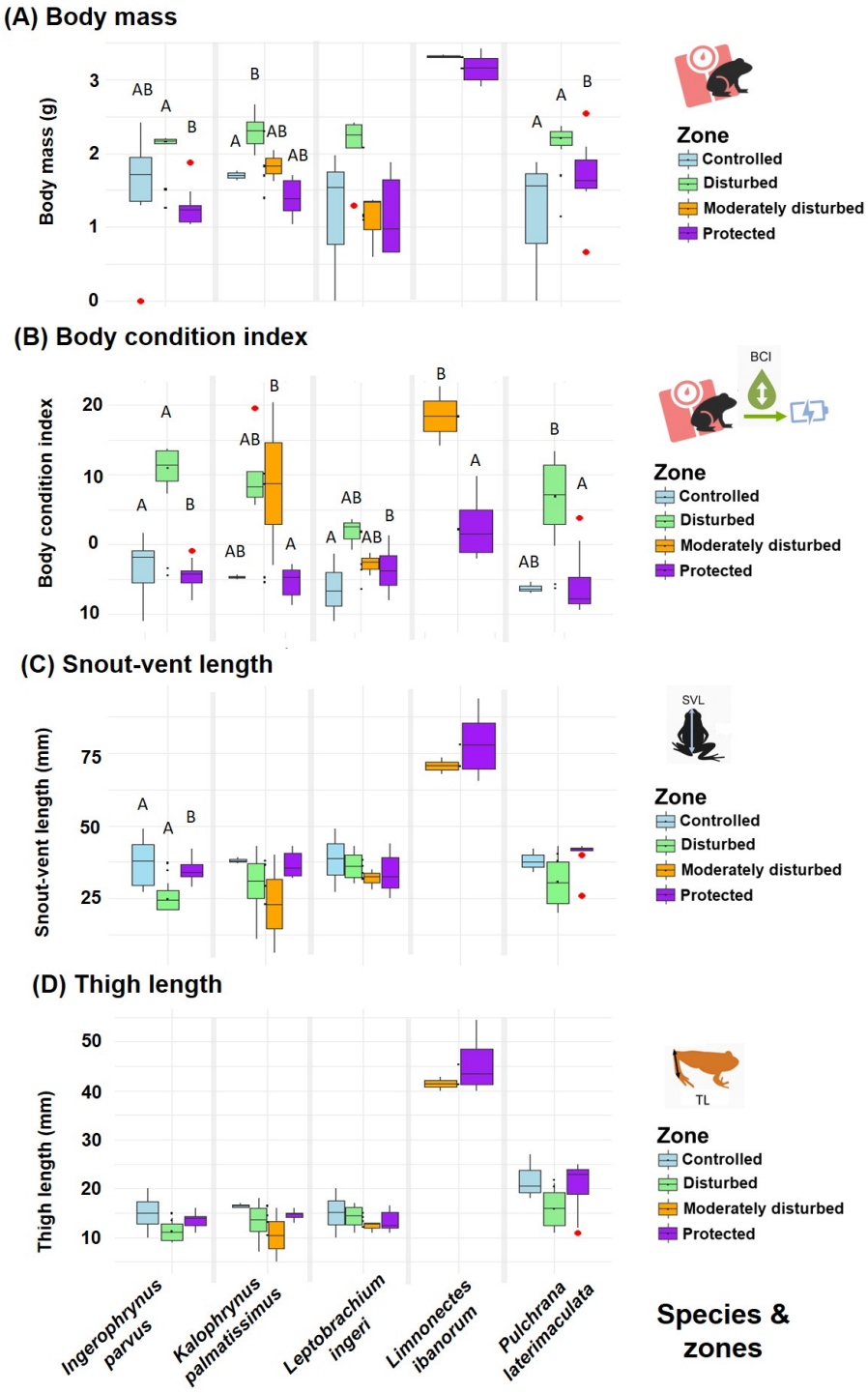

Figure 5 Hidden impacts of disturbance on anuran communities in forests near cities in AHFR, Selangor, Malaysia. Kruskal–Wallis box plots illustrate shifts in body mass, body condition index (BCI), snout-vent length (SVL), and thigh length (TL) across disturbance zones in five most sensitive anuran species from AHFR. (continued on next page…)

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Figure 5 (…continued)
(A–B) illustrate greater variation in physiological traits (body mass and BCI) across zones compared with morphological traits (SVL and TL) depicted in the (C–D). *Ingerophrynus parvus* displayed the most pronounced variation across all traits, while *Kalophrynus palmatissimus* and *Leptobrachium ingeri* showed variation in body condition across moderately disturbed and disturbed zones. While other species displayed relatively stable body condition across disturbance levels. Significant differences based on Dunn's *post hoc* tests ($P < 0.05$) are indicated by different letter groupings (A, B, AB) above boxplots; groups sharing the same letter are not significantly different. Comparisons without letter labels indicate no significant differences among zones.

highlighting differences in SVL, TL, and BCI (Table S19; Fig. 6). The Mann–Whitney-Wilcoxon test revealed significant differences in both Snout-Vent Length ($W = 0$; $P < 0.0001$) and Body Condition Index ($W = 0$; $P < 0.0001$), among the allopatric populations of *L. ibanorum* found both in AHFR and UPMBC.

DISCUSSION

The minimally disturbed zone, composed of the central and eastern regions of AHFR, including the Rasau River, showed the highest species evenness, indicating a balanced distribution of species abundances. Similarly, the protected, controlled, and moderately disturbed zones in AHFR also demonstrated notably high species evenness (Fig. S1). Supporting our hypothesis, despite having the highest species abundance, the disturbed zone in the southern part of AHFR exhibited the lowest species diversity and evenness (Fig. S1), suggesting a community dominated by a few species while others remain rare. Specifically, *Kalophrynus palmatissimus* was the dominant species observed in the disturbed zone, representing 34% of the total species frequency, which is twice the abundance of the second most common species, *Polypedates leucomystax*, at 12.6% (Fig. S1). Both *K. palmatissimus* and *P. leucomystax* were also recorded in forested areas of the minimally and moderately disturbed zones. Particularly for *P. leucomystax*, a highly generalist species (*Hui, 2015*), adaptation to modified habitats may be associated with the availability of food, space, and breeding opportunities, rather than dispersal from forested habitats. Behaviourally, *K. palmatissimus*, like other members of the genus, uses adhesive skin secretions as one of its primary defence mechanisms against disturbances (*Cuta et al., 2022*), which may contribute to greater resistance to environmental change and its persistence in human-modified habitats. Other cosmopolitan species, such as *D. melanostictus* were also frequently observed in disturbed area. In UPMBC, community patterns varied among forest types (Fig. 2), with the campus forest showing lower evenness (0.35–0.45) and greater dominance by a few species, whereas more structurally complex forests such as Nirwana, showed higher evenness (0.55–0.65) and more balanced assemblages, consistent with patterns expected under varying levels of habitat modification.

We found that despite moderate to high diversity, the abundance of some species in the controlled, protected, and undisturbed zones was low. For instance, species such as *Phrynoidis aspera*, *Limnonectes ibanorum*, *L. leporinus*, *Rhacophorus robinsonii*, *Occidozyga lima*, *L. paramacrodon*, *Hylarana glandulosa*, *Ingerophrynus divergens* and *Leptobrachium hendricksoni* were recorded through a single individual in the moderately disturbed zone,

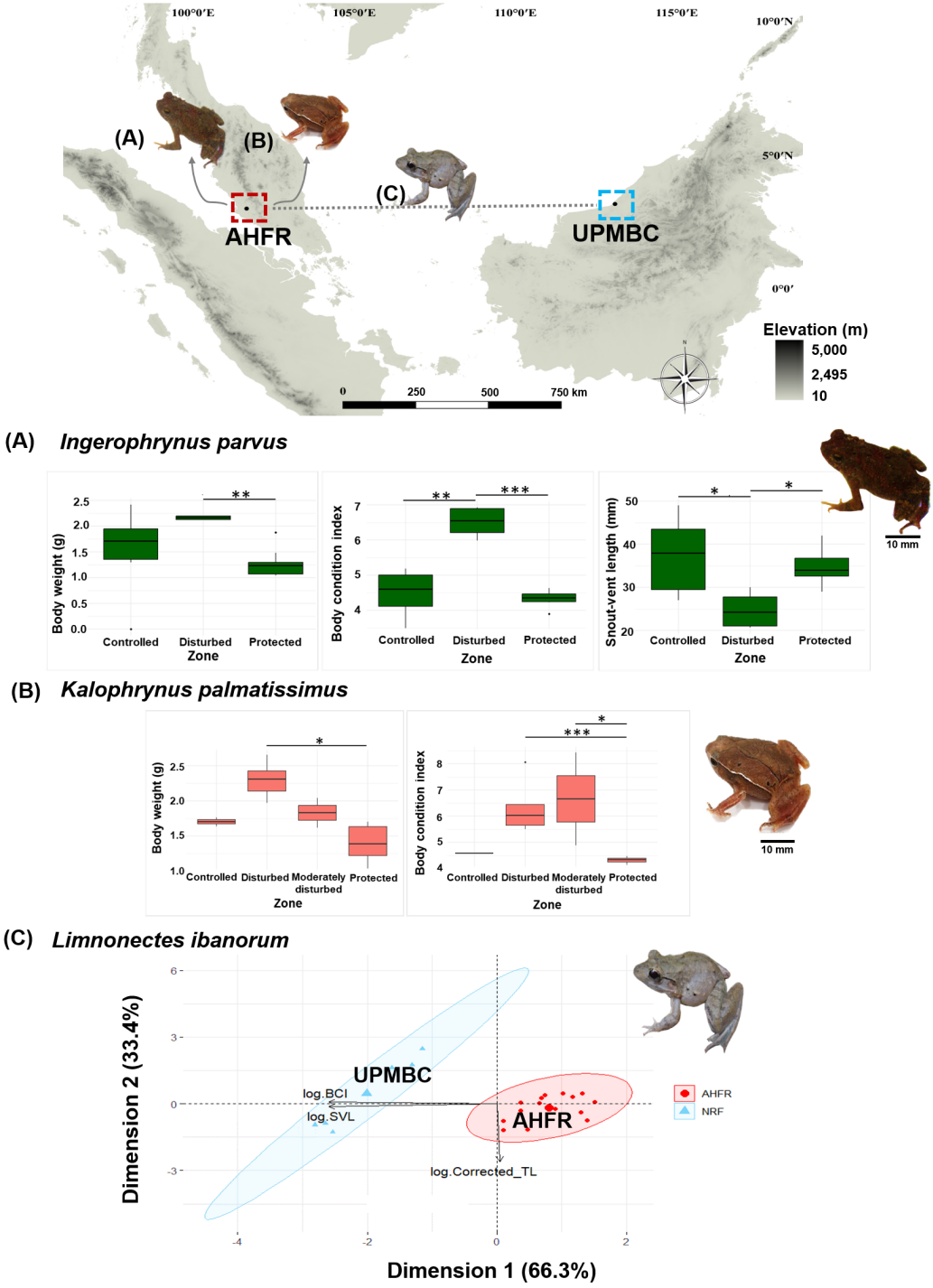

Figure 6 Kruskal–Wallis box plots and Principal Component Analysis biplots of variables attributes to body size and relative fitness of selected anuran species in AHFR and UPMBC. Variables included snout-vent length (SVL), thigh length (TL), body mass and body condition index (BCI). (continued on next page…)

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Figure 6 (…continued)
Box plots show variation in two highly sensitive species across disturbance zones in AHFR: (A) *Ingerophrynus parvus* and (B) *Kalophrynus palmatissimus.* (C) The distribution and PCA biplots illustrate patterns of variation in log-corrected SVL, TL and BCI among allopatric populations of *Limnonectes ibanorum* from AHFR and UPMBC, two geographically separated forests near urban areas. Because AHFR and UPMBC were sampled independently and represent distinct ecological contexts, comparisons between forests are not intended as direct quantitative tests but rather to highlight broad patterns of trait variation. Differences between populations were assessed using the Mann–Whitney-Wilcoxon. The significance levels are indicated by the asterisks as: (*) for $p < 0.05$, (**) for $p < 0.01$, and (***) for $p < 0.001$. Photographs of anurans were taken by Nadia Simon, Muhammad Faris Abdul Aziz, Nur Hidayu Mohd Noor, and Nur Sa'adah Mazlan.

across the streams, trails and swampy areas. Some species are difficult to locate and observe due to their behaviour and characteristics. Some of these species were difficult to observe because of their camouflage, *i.e., I. divergens*. The other species may reflect limited dispersal from the moderately disturbed zone, inhabiting multiple habitats, and just seasonally migrating to the streams of this zone for breeding, from forested areas in the protected zone, *i.e., L. malesianus* and *O. lima.*

The Dicroglossidae family showed the highest number of species in AHFR (Fig. 2B), especially in the protected zone of AHFR (central range). Based on this survey, *Fejervarya moodiei* and *F. limnocharis* serve the highest number of individuals in this family, and found in all trails and streams in all sampling zones compared to the other anuran species that only can be found in one or two sub-locations only. Additionally, the two species showed no significant variation in the body size and BCI across habitats with different levels of human interruption (Fig. 5). These patterns suggest trait variation across environmental contexts, but the dataset does not allow firm inference of adaptive plasticity, including tolerance to saline or peat swamp conditions.

Microhabitat and hydrology quality in the reserve forest are important, as riparian habitats affect anuran species composition in tropical forests (*Ribeiro, Lima & Magnusson, 2012*). Here, rivers and swamps areas are the preferred habitat for some species in the moderately disturbed zone. Therefore, maintaining the hydrological condition in these areas is crucial, especially as they are located at the transition between highly disturbed and less disturbed zones. The two cryptic individuals, *Fejervarya* sp. and *Leptolalax* sp. were found in the swampy and trailing areas within the moderately disturbed zone of AHFR, respectively. The permanent and static swamps in AHFR are unique compared to the peat swamps in Peninsular Malaysia, as they lack both outflow and inflow (*Shamsuddin et al., 2016*). This may increase the vulnerability of swampy habitats in AHFR to human impact, posing a significant threat to the species that inhabit them. In addition, natural vegetation along river streams is essential for providing shelter to riparian species. In the same moderately disturbed zone, only two treefrog species were observed which are *P. leucomystax* (Four-lined Frog) and *Rhacophorus robinsonii* (Robinson's Tree Frog). Behaviourally, these rhacophorid species typically occupy the forest canopy and only move to the forest floor near river streams during the breeding season, likely in response to cooler or more humid conditions. This behaviour makes them difficult to detect outside the

breeding season, particularly *R. robinsonii*. In contrast, *P. leucomystax*, a human-commensal species, is more frequently encountered in modified habitats (*Shahrudin & Jaafar, 2014*).

Humidity emerged as an important microclimatic factor influencing body size in AHFR. Anurans in protected and controlled zones experienced lower humidity, exhibited larger SVL and TL, while those in more humid but moderately disturbed zone showed smaller body size. This pattern suggests that lower humidity, often linked with reduced disturbance and better thermoregulation opportunities, may be associated with increased body size in anurans (*Wells, 2007*). Furthermore, previous study found that soil pH in the moderately disturbed zone was associated with morphological variation in a population of a microhylid species (*Abdul Aziz et al., 2021*). In addition microclimates also impacted the occurrences of some other fauna in the same moderately disturbed zone, such as bats (*Marina et al., 2021*) and upper story birds (*Rosli, Zakaria & Rajpar, 2018*).

Future conservation directions

Our assessment in AHFR emphasises the need to look at both disturbed and moderately disturbed zones, with different conservation priorities. Notably, anurans in moderately disturbed zones showed the smallest body sizes and poorest condition compared to both disturbed and protected zones (Fig. 2), indicating that intermediate disturbance creates particularly stressful conditions that limit growth and reduce fitness. Our findings suggest that moderately disturbed habitats may act as ecological traps, where neither resilience to disturbance nor access to stable resources supports viable populations. While anurans in disturbed zone exhibited slightly better body condition, this may reflect a trade-off between growth and energy storage in more altered environments. The disturbed zone warrants conservation attention due to ongoing habitat degradation and the risk of long-term population decline. These findings are particularly relevant for fragmented tropical landscapes, where mosaics of forest types and disturbance regimes may interact to shape amphibian communities.

In UPMBC, variation in community structure and morphological traits across forest types further indicates that habitat heterogeneity within secondary forests can influence anuran assemblages (Fig. 2). For instance, the campus forest showed lower evenness and higher dominance by a few species, whereas more vegetation-rich forests such as Nirwana supported more balanced assemblages. Although these patterns were not analysed under an explicit disturbance gradient, as in AHFR, they highlight that differences in vegetation complexity and land-use history may also shape community organisation. This suggests that conservation strategies should consider not only disturbance intensity but also variation in forest type and habitat configuration across managed landscapes. All the anurans identified in the undisturbed, controlled, and protected zones in AHFR were typically non-cosmopolitan species that inhabit purely forested areas. This follows the general trend of species composition and distribution in other primary forest in Malaysia, where habitat-generalists such as *Fejervarya* sp. and the human-commensal species, *Duttaphrynus melanostictus* occur in disturbed habitat, while habitat-specialists are more limited to riparian habitat (*Zakaria, BDFH & Norhayati, 2015*). From a conservation perspective, the presence of forest-dwelling anurans exclusively within the controlled and protected

zones is consistent with the potential effectiveness of current management practices. Effort to minimize human contact by monitoring the quality of hydrology especially in the peat swampy areas (*Shamsuddin et al., 2016*), and preserve mosses, plants and animal diversity within the forest (*Hanum, 1999*; *Hanum, Pius & Awang Noor, 1999*; *Damanhuri & Maideen, 2001*), are especially effective in preserving riparian anurans in these areas. Despite some portions of the forest being accessible for education and research purposes (*Ismail, 1999*), ongoing surveillance to restrict human contact in the northern and central ranges of the AHFR should be continued, aiming to preserve the forests in general, and the fauna within them. Overall, conservation strategies in AHFR should therefore address the specific challenges of each zone, ensuring that transitional habitats are not overlooked in efforts to maintain amphibian diversity and ecosystem integrity. This underscores the importance of conserving transitional habitats, where intermediate disturbance may disproportionately affect species persistence and community stability.

For species-specific conservation, notably, *I. parvus* showed the strongest and most consistent inter-zone variation in BCI, including significant differences between populations in both controlled and disturbed, and disturbed and protected zones, making it among the more sensitive species to habitat disturbance in this study. *Kalophrynus palmatisimus* and *L. ingeri* showed relatively large differences in BCI across the zones, suggesting that their body condition is more variable depending on the habitat quality of zone (Fig. 3). Higher BCI values in the controlled zone for *I. parvus* and *K. palmatissimus* indicate more favorable conditions in less altered habitats, whereas *K. palmatissimus* exhibited reduced BCI in the disturbed zone, highlighting potential vulnerability to degradation. In contrast, species such as, *P. laterimaculata* showed minimal variation in BCI across zones, suggesting greater tolerance to environmental change. *Pulchrana baramica* and *L. ibanorum* exhibited higher BCI in protected zones, providing support that undisturbed habitat supported better physiological condition for some species.

Species inhabiting the disturbed areas within the forest, such as the forest base site and campus area in both AHFR and UPMBC, are particularly adaptable to human-modified landscapes. In AHFR, *Ingerophrynus parvus* stood out for its stronger variation in morphological traits across disturbance zones (Fig. 5), indicating a potential sensitivity to habitat modification (Fig. 6A). For *I. parvus* in particular, the SVL, body mass, and BCI varied significantly between the controlled, disturbed, and protected zones, indicating different adaptive responses to the conditions in disturbed and natural landscapes within AHFR. Despite the variations in body sizes and relative fitness for *I. parvus* in both disturbed and protected landscapes could indicate that the species has a degree of adaptability in its physical traits, however, the divergence in traits attributed to body sizes and fitness across the quality of habitats could potentially influence the reproductive success in long term, can promote the reproductive isolation of certain populations, especially when the pattern was priori identified in the toad (*Malone & Fontenot, 2008*). Furthermore, dead leaves within forest floors and open fields are common conditions and habitats for *I. parvus* in secondary forests of Peninsular Malaysia (*Shahrudin & Jaafar, 2012*), underscoring the importance of maintaining the integrity of plant canopy and forest floor across the zones in AHFR. Several species showed lower body condition in moderately disturbed zone,

including *Alcalus baluensis*, *Limnonectes paramacrodon* and *Pulchrana baramica* (Fig. 3). These patterns suggest that some taxa may respond differently to intermediate levels of disturbance, although the present dataset does not allow formal classification of habitat specialization or direct inference of vulnerability. In UMPBC, planted forest supported relatively large-bodied species such as *L.ibanorum*, *Megophrys nasuta* and *Polypedates otilophus.* However, their occurrence in these habitats is interpreted as an association with current forest structure, rather than evidence of habitat dependence. Overall, these results highlight variation in species responses across forest types, but do not permit conclusions regarding habitat specialization or conservation sensitivity.

The divergence in body size and condition between the two populations of *L. ibanorum* occurring in AHFR and UPMBC is likely influenced by geographical isolation (Fig. 6C). The variation between the allopatric populations may also suggest a divergence in environmental and predation pressures between the reserve forests in Peninsular Malaysia and Borneo, contributed by various factors such as topography, vegetation composition, and human activities. For example, the annual pattern of rainfall varies between Peninsular Malaysia and Borneo due to differences in their longitude and the distinct land and ocean distributions (*Sa'adi et al., 2019*), which influence the characteristics of the monsoons (*Wong et al., 2009*). Soil conditions between the two regions can lead to differences in habitat types and availability, which in turn affect the species composition and ecological dynamics. Additionally, different levels of human disturbance, such as logging, agriculture, and urbanization, can lead to variations in environmental conditions, which in turn can affect predation pressures (*Folt & Guyer, 2021*). Future studies should explore ecological and genetic aspects to confirm geographic variations in body size and condition of this species. Specifically, incoming study should focus on potential adaptational responses to anthropogenically-linked zones. Moreover, clarifying patterns of ecological niche divergence between the two allopatric reserve forests may elucidate factors influencing their comparative species variation.

Limitation of the study

Several limitations should be considered when interpreting the results of this study. This study integrates data from two forest systems, AHFR in Selangor and UPMBC in Sarawak, which differ in geographic setting, forest structure, survey period, and sampling context, and were therefore analysed and interpreted independently rather than as direct quantitative comparisons. As the dataset was compiled from long-term surveys conducted across different forest types and years, variation in sampling effort, seasonal timing, and environmental conditions may influence detectability and relative abundance estimates. To reduce potential bias associated with uneven sampling effort, we applied sample-based rarefaction and standardised encounter rates using CPUE, allowing more comparable estimates across zones and forest types. Seasonal categories used in the analyses refer to relatively wetter and less-wet sampling periods rather than discrete climatological seasons, reflecting the constraints of field-based sampling. Visual encounter surveys may introduce detectability bias, as larger-bodied or more conspicuous species, particularly in structurally open habitats, are more likely to be detected than cryptic species in dense

vegetation. Therefore, CPUE reflects relative abundance rather than absolute abundance, and community estimates may be influenced by differences in detectability among habitats and species. Our analyses of body size and condition were conducted at the community level, such that observed patterns may partly reflect differences in species composition among habitats rather than within-species responses. Finally, the observational nature of the dataset limits causal inference regarding the mechanisms underlying variation in body size and condition.

CONCLUSION

Human disturbance and habitat modification both influence anuran communities, body condition, and morphological variation in urban-adjacent forests. In AHFR, disturbance across zones was associated with reduced diversity and evenness, alongside shifts in body size and condition, with moderately disturbed areas showing the lowest body size and condition. In UPMBC, community patterns varied among forest types, with the campus forest showing lower evenness and stronger dominance by a few species, whereas the more structurally complex, Nirwana forest supported more balanced assemblages. We suggest that conservation efforts should not focus solely on minimally disturbed habitats, but also consider areas with intermediate levels of disturbance or modification, as these may represent vulnerable systems where reduced body size and condition indicate potential stress on anuran populations.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Siti N. Othman conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Marina Mohd. Top @ Mohd. Tah conceived and designed the experiments, authored or reviewed drafts of the article, project manager, and approved the final draft.
- Deyatima Ghosh conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Nadia Simon performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Muhammad Faris Abdul Aziz performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Nur Hidayu Mohd Noor performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Nur Sa'adah Mazlan performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Amaël Borzée conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The surveys were carried out in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines of Universiti Putra Malaysia (reference no: UPM/IACUC/AUP-R007/2018).

Data Availability

The following information was supplied regarding data availability:

The data is available in the Supplemental File.

R scripts used for the statistical analyses are available at Zenodo: OthmanSN. (2026). OthmanSN/Malaysian-anurans-mixed-models: Reproducible analysis for anuran mixed models (PeerJ submission) (v1.0). Zenodo. https://doi.org/10.5281/zenodo.19664521.

The data is available at Mendeley Data: Othman, Siti N (2026), "Anurans in forests near cities in Peninsular Malaysia and Malaysian Borneo reflect the impact of habitat disturbance on species' diversity and relative fitness", Mendeley Data, V3, DOI: 10.17632/d7mrsx9hr9.3.

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