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Cover: Freshly emerged Footman Moth *Nepita conferta* from the cocoon on a brightly painted wall in the Nilgiris. Digital art on Procreate. © Aakanksha Komanduri.

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ARTICLE

Morphometry and feeding notes of an endemic frog *Amolops spinapectoralis* (Amphibia: Ranidae) from Hue City, central Vietnam

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Abstract: This study presents the first record of the endemic ranid frog *Amolops spinapectoralis* in Hue City, Vietnam. Notably, the specimens collected exhibit cylindrical spines, a distinct morphological feature differing from the conical spines originally described, highlighting the species' local morphological diversity. Given the limited ecological data on this species, we analyzed its morphological characteristics and investigated the correlation between body size and feeding ecology, as well as sexual variation in diet composition and prey diversity. We identified 507 prey items across 19 prey categories. The dominant prey taxa included Orthoptera, Coleoptera, Diptera, Formicidae, Isoptera, insect larvae, and Hymenoptera, which collectively accounted for 71.16% of the frequency of occurrence, 68.24% of the total prey number, and 66.89% of the total volume, with a relative importance index of 68.77%. Furthermore, prey mass was positively correlated with temperature and relative humidity, independent of rainfall.

Keywords: Cylindrical spines, diet composition, feeding ecology, morphological feature, ranid frog, prey diversity, prey moss, sexual variation.

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Author contributions: Loi Duc Duong, the lead author of the paper, along with Giang Van Tran, who worked on the project DHH2023-03-181 and participated in processing the nutritional ecological characteristics; and Nghiep Thi Hoang, a member who performed the analysis of morphological characteristics of the rock-clinging frog (*Amolops*).

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INTRODUCTION

Spinyback Torrent Frog *Amolops spinapectoralis* Inger, Orlov & Darevsky, 1999 is a ranid frog endemic to Vietnam (Nguyen et al. 2009). However, it has now also been found in Laos and presumably in adjacent northeastern Cambodia (Frost 2025). In Vietnam, this species has been recorded in Gia Lai, Da Nang, Quang Nam, Quang Ngai, Kon Tum, Phu Yen, and Quang Nam Provinces (Frost 2025).

Recent studies on *Amolops* in Vietnam and around the world have mainly focused on taxonomy and have not focused on comprehensive and complete research on the biological, morphological, and ecological characteristics of this species group. This article focuses on studying the morphological characteristics and nutritional ecology of this species in Hue City.

We study the morphological characteristics and feeding ecology of *A. spinapectoralis* in Hue City, Vietnam. Specifically, our research focused on:

(1) Morphological characteristics of *A. spinapectoralis* living in Hue City;

(2) Diet composition and foraging strategy;

(3) Variation between males and females in prey composition;

(4) Variation between locations and seasons in feeding ecology;

(5) Correlations between morphological characteristics and prey; and

(6) Influence of environmental factors.

This study hypothesized that adult females of *A. spinapectoralis* possess larger head sizes than males. To test this, variation in prey consumption was explored across sites, seasons, and sexes. The foraging strategy was predicted to align with the ‘sit-and-wait’ model. Additionally, the species’ foraging ecology was examined to estimate seasonal dietary variation and test the hypothesis of a positive correlation between body size, prey size, and diet. Specifically, mouth width was hypothesized to influence consumed prey size, with larger frogs predicted to consume larger prey.

MATERIAL AND METHODS

Study Site

This study was conducted in the tropical rain forests of Hue City 15.991^0 – 16.741^0 N,107.015^0–108.215^0 E, central Vietnam (Figure 1). On 1 July 2025, Thua Thien Hue Province was officially reorganized into a centrally run city named Hue City. According to Resolution No. 1675/NQ-UBTVQH15 (issued by the National Assembly Standing Committee on 20 June 2025), the new city comprises 40 commune-level administrative units, including 21 wards, and 19 communes.

The study area features a subtropical monsoon climate dominated by montane rain forests (700–1,400 m elevation) and cloud forests (>1,450 m elevation). There is no dry season; instead, the year comprises a light rainy season (January–April), an auxiliary rainy season (May–August), and a main rainy season (September–December). Annual rainfall ranges from 3,970–4,281 mm with an average temperature of 23.8–25.2 °C (Figure 2). (Nguyen et al. 2004; Ngo et al. 2012, 2013).

Stomach contents of *A. spinapectoralis* were collected at three sites in Hue City: (1) Do Quyen Stream (Phu Loc; 1,096 m elevation; N = 91); (2) Thuong Lo Stream (Khe Tre; 334 m elevation; N = 87); and (3) A Pat Stream (A Luoi 4; 1,021 m elevation; N = 83) (Figure 3).

Morphometric Analyses

A total of 261 individuals (130 males and 131 females) were collected for dietary studies. Of these, morphological data were recorded on only 235 individuals (84 males, 110 females, and 41 sub-adults). After completing stomach sampling and morphological measurements, the individuals were released back into the wild. All measurements were taken with a caliper to the nearest 0.01 mm following Duong & Ngo (2022), Pham et al. (2015, 2023a).

Abbreviations

SVL—snout-vent length, distance from tip of snout to anterior margin of vent | HL—head length, from tip of snout to rear of jaws | HW—Head width, horizontal line at posterior end of the mandible | SL—snout length, distance from anterior corner of eye to tip of snout | ED—eye diameter, from anterior corner to posterior corner of eye | EN—eye to nostril, distance from anterior corner of eye to the nostril | IND—internarial distance, least distance between upper eyelids | IOD—minimum distance between upper eyelids | MW—mouth width, horizontal line at the posterior angle of the jaw | TD—tympanum diameter, horizontal width of the tympanum at its widest point | TL—tibia length, from knee to heel | FL—foot length, from proximal end of inner metatarsal tubercle to tip of fourth toe | TFL—length of foot and tarsus, from tibiotarsal joint to tip of fourth toe | finger or toe length—distance between posterior margin of most proximal subarticular tubercle or crease of articulation and tip of finger or toe | width of disc on finger or toe—greatest width of terminal disc on finger

Figure 1. Map of Hue City, Vietnam, showing the geographical location and locations where we sampled *Amolops spinapectoralis* (blue circles): 1—Phu Loc commune (the old place name is Loc Tri commune, Phu Loc District) | 2—Khe Tre commune (the old place name is Huong Loc commune) | 3—A Luoi 4 commune (the old place name is A Roang commune)—location updated per Hue City's administrative map (from 1 July 2025).

or toe | thigh length—from the center of knee to the center of the hind limb insertion | foot length—from the base of the inner metatarsal tubercle to the tip of the fourth toe. Some ratios of head morphology and ratios of head measurements and tibia length compared to SVL, such as HL/HW, HL/SVL, HW/SVL, MW/SVL, and TL/SVL. To measure body mass (BM), we used electronic scales (Kern, Germany) accurate to 0.01 g.

Stomach Content Collection

We analyzed the nutritional ecology of 261 individuals (131 females, 130 males) from three localities in Hue City via stomach flushing. Samples were evenly distributed across three seasons (N = 87 each): low-rainy (January–April), secondary rainy (May–August), and main rainy (September–December). Gastric lavage was performed to collect stomach contents from frogs without causing harm (Solé et al. 2005; Pham et al. 2023a). To avoid oesophageal or gastric injury, especially in smaller frogs, we used tea strainers, a fine mesh sieve, two 60 ml syringes, and soft silicone tubing. Each frog underwent a single gastric lavage following the American Society of Ichthyologists and Herpetologists' animal care guidelines (Beaupre et al. 2004). Filtered stream water from the capture sites was used during the process. Frogs were monitored post-lavage and released within 30 minutes if found to be in good condition. Specimen sex was determined by examining the vocal sacs and gonads (Duong & Ngo 2022; Pham et al. 2023b).

All stomach contents were initially preserved in 95% ethanol for later laboratory analysis. Following examination, specimens and stomach contents were transferred to 70% ethanol and deposited in the Amphibian Collection at the University of Education, Hue University, Vietnam. Prey from each stomach sample was sorted and identified to the lowest possible taxonomic level, primarily to order, with identification made by family when possible. For taxonomic identification of invertebrates, we followed Thai (2003) and Johnson & Triplehorn (2005). Prey identification was conducted under a microscope (Olympus SZ 700) and stereo microscope (XT-203) using various keys (Millar et al. 2000; Johnson & Triplehorn 2005; Brusca et al. 2016). All individuals were released back into the wild after stomach contents were collected and morphological data were measured.

We measured the length and width (at the widest central part of the body) of each prey item using a digital caliper, accurate to 0.01 mm, or made the best estimate for incomplete items. Both animal and plant matter were classified as food. Unidentified materials, such as digested insects in the diet of *A. spinapectoralis*, were categorized as unidentified. Non-food materials, including sand and rocks, were excluded from the analysis. We calculated the volume fraction of each prey item and unidentified material, estimating the volume (V) using the formula for an expanded sphere with $\pi = 3.14159$ (Magnusson et al. 2003; Biavati et al. 2004; Valderrama-Vernaza et al. 2009; Caldart et al. 2012; Ngo et al. 2013; Pham et al. 2023a):

$$V = \frac{4\pi}{3} \text{x} \left(\frac{\text{length}}{2}\right) \text{x} \left(\frac{\text{width}}{2}\right)^2, \text{mm}^3$$

We used the relative importance index (IRI) to assess the significance of each prey species in the feeding ecology of *A. spinapectoralis*. The IRI offers a comprehensive estimate of prey consumption by combining three key components, providing a more complete evaluation than considering any single component. The formula for calculating the IRI is as follows (Biavati et al. 2004; Leavitt & Fitzgerald 2009; Ngo et al. 2013; Pham et al. 2023a):

$$\text{IRI} = \frac{\%\text{F} + \%\text{N} + \%\text{V}}{3}$$

The IRI represents the importance index for each food item, where F is the frequency of stomachs containing a specific prey item, N is the total number of prey items counted, and V is the volume of the corresponding prey. This index provides a more biologically meaningful assessment, offering a more realistic evaluation of prey significance compared to individual assessments of frequency (F), number (N), or volume (V) alone (Leavitt et al. 2009; Ngo et al. 2014). To estimate the influence of sex and season on the foraging ecology of *A. spinapectoralis*, we calculated an IRI for prey types, based on individual stomach contents and their importance values.

We use reciprocal Simpson's heterogeneity index, 1/D, to calculate dietary heterogeneity:

$$D = \frac{1}{N(N-1)} \sum n_i(n_i - 1)$$

n_i is the number of prey items in the ith prey category and N is the total number of prey categories (Krebs 1999).

To estimate prey evenness, we used Shannon's index of evenness. Evenness is calculated from the equation:

from the equation: $$\text{J}' = \frac{\text{H}'}{\text{H}_{\max}} = \frac{\text{H}'}{\ln\text{S}},$$

The maximum diversity (H_{max}) that could occur is that which would be found in a situation in which all taxa had equal abundance ($H = H_{max} = \ln S$), S is the total number of prey taxa, and H' is the Shannon-Weiner index of taxon diversity. Here's the complete formula for calculating the Shannon-Wiener diversity index (H'):

$$H' = -\sum (p_i \, x \, ln(p_i))$$

Where *pi* is the proportion of total food items belonging to the taxon relative to the ith total food items in the sample (Magurran 2004; Pham et al. 2022). This index measures species diversity in a community, with higher values indicating greater diversity.

Foraging strategy

During three seasons, we observed foraging activity of *A. spinapectoralis* on the waterfalls. Each individual encountered was monitored for approximately 10 minutes, with movements and corresponding behaviours recorded every minute. These data were used to analyze and compare two main activity patterns: the "widely foraging" pattern and the "sit-and-wait" pattern. A total of 360 minutes of observation was conducted (30 minutes per survey, with 1 observation being 10 minutes).

Data Collection

Nighttime surveys were conducted along 2.0–3.0 km stream transects to hand-collect specimens for morphological measurement and non-lethal stomach flushing. All specimens were released back into their natural habitat immediately after processing. For each capture, we recorded environmental variables (date, time, temperature, humidity) and determined the species' distribution in Hue City using a GPS device (Garmin 64S; Garmin USA; Taiwan; WGS 84).

Statistical Analysis

Statistical analyses were performed using SPSS 22 with a significance level of $P \le 0.05$, and data are presented as mean ± SD unless otherwise noted. Sexual size dimorphism (based on SVL) was tested using two-way ANOVA, followed by ANCOVA for other morphological traits (head, BM, SVL, MW) (Rosner 2010; Zar 2010; Ngo et al. 2014). We used one-way ANOVA to compare stomach counts and prey metrics across sexes, seasons, and localities; analyses were stratified by sex where significant sexual dimorphism occurred. Finally, multiple linear regression was employed to examine correlations between body size (SVL, BM, MW) and prey dimensions, as well as the effects of climatic factors (precipitation, temperature, humidity) on prey mass

(Ngo et al. 2014; Pham et al. 2022).

Genetic Analysis

The study was conducted using molecular data and phylogenetic analysis of the CO1 (Cytochrome c Oxidase Subunit 1) gene, which is located on the mitochondrial DNA (mtDNA) of *A. spinapectoralis* in Hue City. The DNA sequence after sequencing was edited and quality checked using BioEdit version 5.0.9 software. The edited sequences were then compared with the NCBI database using the BLAST tool to determine the level of similarity with known sequences (Benson et al. 2017).

RESULTS

During this study, our collections of *Amolops spinapectoralis* spanned the whole 12-month period, from February 2023 to January 2024, wherein the species was recorded at various locations in the Hue City (see Figure 1).

Genetic findings

The research sample coincided with the sequence of the standard sample *A. spinapectoralis* (voucher KIZ013694) stored on GenBank (MN953768.1) with a divergence of 0.22%. Comparison with the GenBank database showed that the obtained sequence had a high similarity with the species *A. spinapectoralis*.

Morphometry

The analysis results showed that, in the study area, the head length/body length ratio in adult females (n = 110) was significantly larger than that in adult males (n = 84) (Table 1).

Morphological characters (Image 2): Head is slightly longer than it is wide (female: HL/HW = 1.12 ± 0.13; male: HL/HW = 1.16 ± 0.14); blunt snout that projects forward relative to the lower jaw; nostrils are rounded and positioned closer to the eyes than to the snout tip (female: SL 12.74 ± 2.56; EN 7.02 ± 1.17; NS 3.29 ± 0.88; male: SL 11.18 ± 3.28; EN 5.92 ± 1.17; NS 2.94 ± 0.95); internasal distance is larger than both the interorbital distance and upper eyelid width (female: IOD 1.34 ± 0.46; IN 6.09 ± 1.25; male: IOD 1.38 ± 0.98; IN 5.98 ± 4.36); eyes are large and prominent, with a diameter larger than the tympanic membrane (female: ED 6.17 ± 2.05; TD 1.91 ± 0.97; male: ED 5.26 ± 0.96; TD 1.68 ± 0.67). A visible skin ridge is present above the tympanum; vomerine teeth are short and arranged in a V shape, and the tongue is slightly forked posteriorly; males have a vocal sac and white, cylindrical pectoral spines. Forelimbs: forelimbs lack swimming webs and with suction discs with a circum-marginal groove on the disc of the first finger; adult males possess ivory-white spinous pads at the base of the index finger. The relative lengths of the fingers are I<II<IV<III. Hind limbs: Hind digit discs are smaller than those of the forelimbs. The relative lengths of the hind digits are I<II<III<V<IV; swimming membranes are wide, extending fully to the discs of the hind digits, with no external tubercles present. Skin: Dorsal skin is rough with small pimples, while the ventral skin is smooth. Colour in life: Head and body are grey-blue or olive-grey, with dark black streaks and spots forming a network; eyes sockets are dark brown, and the flanks are covered in white pimples; ventral surface is white, with females showing yellow streaks or spots on the flanks in breeding season.

The snout-vent length (SVL) ranged 39.73–56.64 mm in adult males (n = 84), 39.73–556.64 mm in adult females (n = 110), and 15.91–337.95 mm in subadults (n = 41). On average, both SVL and body weight (BW) of males were noticeably smaller than those of females (Table 1). The largest female had an SVL of 56.64 mm, while the largest male measured 52.48 mm (Table 1). The mean SVL of adults did differ significantly between sexes (F = 135.148, Sig. < 0.001). The SVL did vary significantly across seasons, temperatures, and humidity (season: F = 8.256, Sig. < 0.001; temperature: F = 5.373, Sig. < 0.001; humidity: F = 7.012, Sig. < 0.001). There was no significant difference in SVL among localities (F = 1.718, Sig. = 0.182).

The basic head size measurements in Hue city, analyzed through a two-factor ANOVA for males and females, yielded significant results. For head length, males measured an average of 14.83 ± 1.59 mm, while females measured 16.85 ± 1.76 mm (F = 6.212; Sig. = 0.000). Head width showed a similar trend, with males at 12.94 ± 1.5 mm and females at 15.1 ± 1.75 mm (F1,59 = 5.809; Sig. = 0.000). Snout length (SL) averaged 11.18 ± 3.28 mm for males and 12.74 ± 2.56 mm for females (F = 3.065; Sig. = 0.003). For mouth width, males averaged 13.89 ± 2.12 mm, compared to 16.41 ± 1.63 mm for females (F = 7.868; Sig. = 0.000). Overall, adult females exhibited significantly larger head sizes than males. Linear regression analysis indicated that body mass increased with body length in both sexes. When adjusting for SVL, adult females still showed larger measurements than males across head length (HL: F = 554.214, Sig. = 0.000), head width (HW: F = 393.860, Sig. = 0.000), and mouth width (MW: F = 272.103, Sig. = 0.000). Furthermore, adult females had a significantly

Table 1. Morphological characteristics of *Amolops spinapectoralis* from Hue City, including weight (g), measurements (mm), and proportions of the specimens.

Character	Adult females (n = 110)		Adult males (n = 84)		Subadults (n = 41)	
Measurements	min–max	mean ± SD	min–max	mean ± SD	min–max	mean ± SD
SVL	39.73–56.64	48.97 ± 4.64	32.46–52.48	41.04 ± 5.92	15.91–37.95	30.71 ± 5.6
HL	12.58–20.34	16.85 ± 1.76	10.39–17.94	14.83 ± 1.59	8.41–18.96	12.25 ± 2.0
HW	10.03–18.1	15.1 ± 1.75	9.72–16.26	12.94 ± 1.5	6.6–16.81	10.57 ± 2.06
SL	3.96–16.85	12.74 ± 2.56	5.27–20.83	11.18 ± 3.28	2.38–13.63	8.63 ± 2.35
ED	3.53–15.84	6.17 ± 2.05	3.47–7.83	5.26 ± 0.96	1.53–6.6	4.04 ± 1.14
IOD	0.47–2.26	1.34 ± 0.46	0.36–6.9	1.38 ± 0.98	0.34–6.97	1.67 ± 1.64
TD	0.74–7.63	1.91 ± 0.97	0.45–4.99	1.68 ± 0.67	0.53–4.08	1.52 ± 0.79
ET	0.83–18.94	3.34 ± 2.27	1.16–4.02	2.69 ± 0.7	1.2–3.62	2.07 ± 0.56
TL	16.1–25.89	22.22 ± 2.36	2.22–25.08	19.67 ± 3.56	10.79–23.01	15.33 ± 3.23
FL	11.33–31.18	24.47 ± 3.24	15.52–27.32	21.36 ± 2.83	22.14–12.02	16.6 ± 3.06
HND	5.15–23.47	14.28 ± 2.59	8.86–17.74	12.34 ± 1.84	7.03–15.72	9.89 ± 2.03
PL	5.71–28.18	21.12 ± 3.91	12.26–27.27	18.46 ± 2.89	3.34–21.2	13.93 ± 3.65
IN	0.03–9.13	6.09 ± 1.25	2.94–33.12	5.98 ± 4.36	3.5–7.57	4.62 ± 0.85
P	8.4–82.78	18,52 ± 10,02	5.1–18.71	10.92 ± 3.09	0.99–9.36	4.3 ± 2.05
NS	1.3–6.06	3.29 ± 0.88	1.24–6.84	2.94 ± 0.95	1.6–3.77	2.53 ± 0.61
EN	2.54–8.69	7.02 ± 1.17	2.56–8.73	5.92 ± 1.17	3.27–7.5	5.79 ± 1
ML	4.37–16.52	8.96 ± 1.89	4.46–9.91	7.41 ± 1.27	4.44–9.32	6.41 ± 1.53
PL-4	5.9–24.28	15.69 ± 3.15	5.93–19.38	13.99 ± 2.27	6.3–16.84	10.42 ± 2.48
IML	2.05–6.32	5.04 ± 0.96	2.49–8.65	4.54 ± 1.07	2.7–6.38	3.76 ± 0.92
MW	11.31–19.03	16.41 ± 1.63	4.83–18.15	13.89 ± 2.12	6.44–19.08	11.01 ± 2.53
HL/HW	0.82–1.47	1.12 ± 0.13	0.72–1.44	1.16 ± 0.14	0.04–0.37	0.14 ± 0.15
IO/HL	0.03–0.14	0.08 ± 0.03	0.02–0.53	0.1 ± 0.08	0.04–0.37	0.14 ± 0.15
ED/HL	0.21–0.94	0.37 ± 0.12	0.24–0.49	0.36 ± 0.06	0.14–0.4	0.33 ± 0.07
IN/HL	0–0.18	0.12 ± 0.03	0.07–1.44	0.16 ± 0.19	0.31–0.52	0.38 ± 0.05
HL/SVL	0.28–0.41	0.35 ± 0.03	0.24–4.09	0.4 ± 0.41	0.34–1.19	0.42 ± 0.18
TL/SVL	0.35–0.52	0.45 ± 0.04	0.05–4.39	0.52 ± 0.43	0.37–1.45	0.52 ± 0.22
MW/SVL	0.28–0.4	0.34 ± 0.02	0.11–3.85	0.38 ± 0.39	0.28–1.2	0.38 ± 0.19

SD—Standard deviation | for other abbreviations see Materials and Methods.

greater body mass than their male counterparts (BM: F = 173.416, Sig. = 0.000).

In terms of body mass, adult females were significantly larger than adult males. The average body mass for females was 18.52 ± 10.02 g (range: 8.4–82.78 g), while males averaged 10.92 ± 3.09 g (range: 5.1–18.71 g). This difference was statistically significant (F = 1.940; Sig. = 0.000), indicating that female body mass is notably greater than that of males.

Figure 4A shows a strong correlation between head length and body length; individuals with longer bodies also have longer heads in both sexes. The regression coefficients indicate that this relationship is stronger in females ($R^2 = 0.1366$) than in males ($R^2 = 0.4404$). Figure 4B shows that body length is positively associated with head width; the correlation is higher in females ($R^2 = 0.623$) than in males ($R^2 = 0.3339$).

Figure 4C indicates a similar positive correlation between body length and mouth width; again, the relationship is stronger in females ($R^2 = 0.6192$) than in males ($R^2 = 0.1953$). The average body length of adult females is also significantly larger than that of adult males.

Figure 5A shows that the body mass and mouth width of *Amolops spinapectoralis* are closely related. Linear regression analysis shows that the larger the mouth width, the greater the increase in mass in both males and females. Through the regression coefficient,

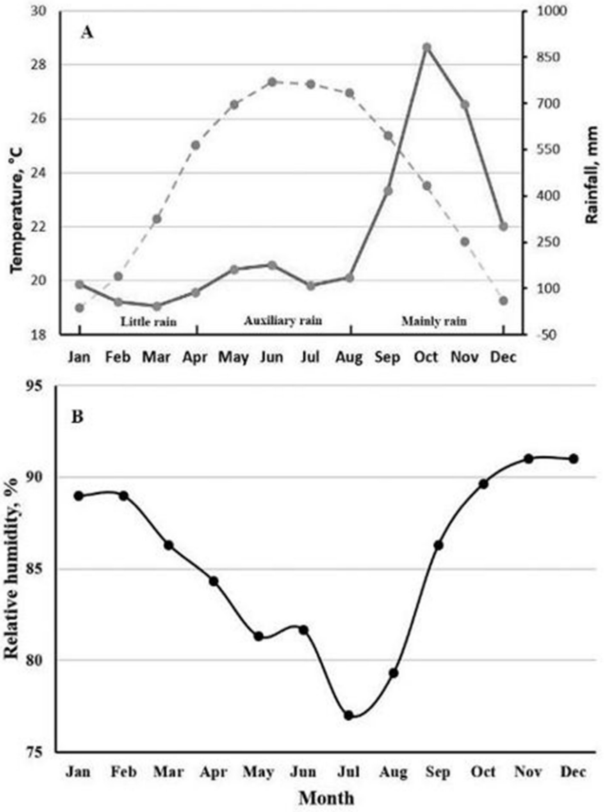

Figure 2. A—Monthly mean rainfall and temperature in the study area. Data correspond to the monthly mean temperature (broken line, in °C) and precipitation (solid line, in millimeters) over the last 20 years | B—Monthly mean relative humidity (solid line, in%) in the study area over the last 20 years. Data recorded from a statistic of the climatic hydrology characters of Hue City (Nguyen et al. 2004).

the relationship between BM and MW of males is closer than that of females (regression coefficient $R^2 = 0.1899$ in females; $R^2 = 0.4487$ in males); through Figure 5A, we see that when males and females have the same mouth width, the mass of females is larger than that of males, which partly shows that the nutritional needs of females are greater than that of males.

Figure 5B shows that body length and body mass are closely related; the larger the body length, the greater the body mass; through the regression coefficient, we see that the relationship between SVL and BM in males is slightly closer than in females (regression coefficient in females $R^2 = 0.1533$; males $R^2 = 0.1899$). Figure 5B also shows that with the same SVL size, the female's mass is larger than that of the male.

Dietary Observations

The majority of the time, 310 minutes (86.1%), was spent lying and waiting, which aligns with the "sit and wait" behaviour. The time spent moving was 23 minutes (6.39%), while hunting activity accounted for 25 minutes (6.94%). The remaining time was devoted to other behaviours, such as mating, escaping predators, and miscellaneous activities. Of the total, 86 stomachs (33%) were empty; thus, 175 stomachs containing food were retained for dietary analysis. The main food components were grasshoppers, beetles, insect larvae, flies, and ants with IRI of 16.17, 13.18, 10.93, and 9.12, respectively,

Image 1. Habitat of *Amolops spinapectoralis* at sampling sites in Hue City, Vietnam: A—A Luoi 4 commune | B—Khe Tre commune | C—Phu loc commune | D—Photographs of the habitat where the frogs were documented. © Loi Duc Duong.

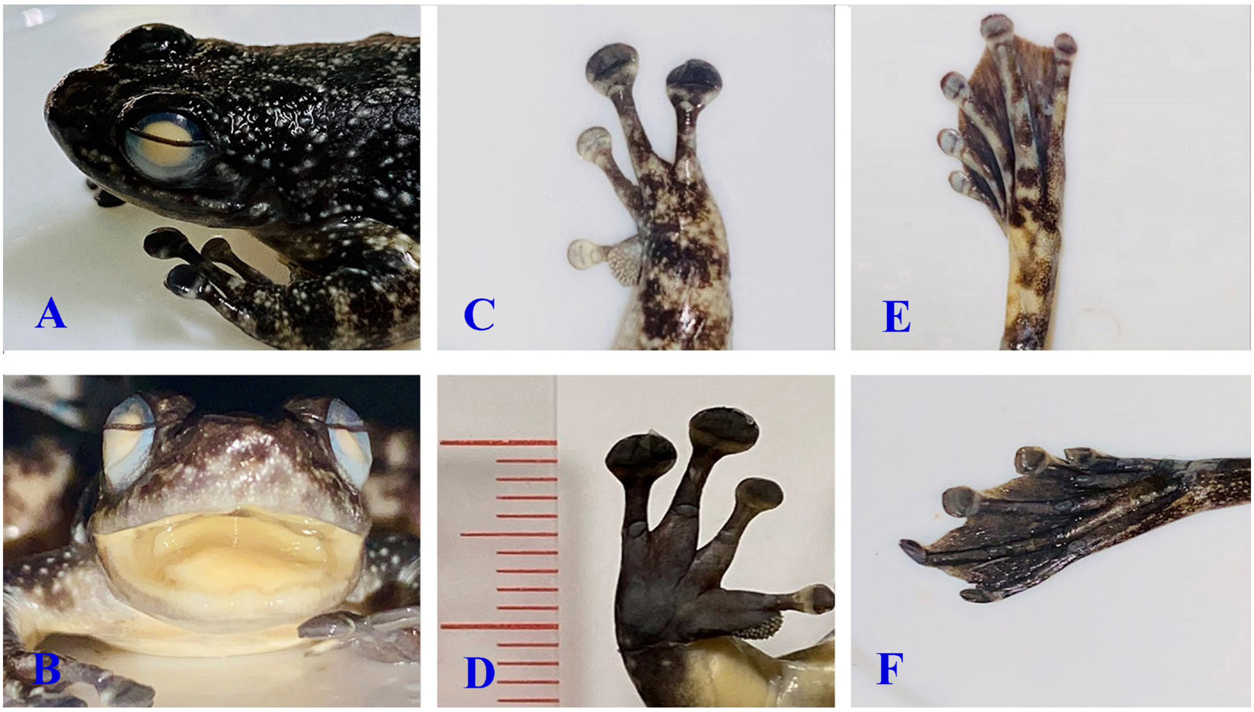

Image 2. Head, hand, and foot of *Amolops spinapectoralis* (male). Head: A—lateral view | B—oral cavity | Hand: C—dorsal view | D—ventral view | Foot: E—dorsal view | F—ventral view. © Loi Duc Duong.

which are common insect orders in fast-flowing waterfall environments. The diet comprised 18 animal prey categories (all insects), one plant category (consisting of seven species, primarily mosses), and 16 unidentified organisms. Traces of sand and rocks were also present, but no anthropogenic materials (e.g., plastics) were found. Prey measurements were as follows: mean length 8.93 ± 4.49 mm (range: 1.25–29.93 mm); mean width 3.39 ± 2.11 mm (range: 0.4–13.15 mm); and mean volume 91.97 ± 198.26 mm^3 (range: 0.75–1873.21 mm^3) (see Table 2).

Surveys at three locations in Phu Loc commune (Bach Ma National Park), Khe Tre commune (the old place name is Huong Loc commune), and A Luoi 4 commune (the old place name is A Roang commune) showed that the most abundant prey were Orthoptera, Coleoptera, Diptera, Formicidae, Isoptera, insect larvae, and Hymenoptera, accounting for 71.16% of the frequency of occurrence, 68.24% of the number of prey, and 66.89% of the total volume, with a relative importance index of 68.77% (Table 2). Based on the relative importance index, Coleoptera, Formicidae, insect larvae, and Diptera were the most important prey items, while Araneae, Blattodea, Gryllidae, Julidae, Lepidoptera, and Neuroptera were the less important prey items, accounting for 7.47% of the total prey items with a relative importance index of 6.52% (Table 2). The remaining prey items, such as Dermaptera, Hymenoptera, Isoptera, and Trichoptera, were intermediate in importance, with a relative importance index of 16.58% (Table 2). In addition, We found seven different types of plants in the stomach (these were mosses and small plants growing on the cliffs of the flowing waterfall) with a relative importance index of 6.11 % (Table 2). Some sand and some stones were also found in the stomach, but it is likely that they were accidentally swallowed along with the prey. Therefore, we excluded them from the analysis.

Male vs. female variations in prey composition

We identified a total of 507 prey items from the 175 stomachs containing food. Females consumed 274 items and males consumed 233 (F = 2.932, Sig. = 0.089, $p > 0.05$). On average, each individual consumed 2.66 prey items (range: 0–10).

In Figure 6, there are seven common food types; based on the IRI importance index, we can see that the food requirements of females are Coleoptera, Formicidae, insect larvae, and Isoptera higher than those of males in a total of 261 stomachs surveyed. On the contrary, in males, the food requirements of Orthoptera prey types are higher than those of females. Adult females (18 prey species) consumed more diverse prey species than adult

Table 2. Dietary composition of *Amolops spinapectoralis* in Hue City.

Prey category	Frequency (F)		Count (N)		Volume (V)		IRI
	F	%F	N	%N	V	%V	
Araneae	4	1.08	4	0.79	126.51	0.27	0.71
Blattodea	4	1.08	6	1.18	310.65	0.66	0.97
Coleoptera	45	12.13	66	13.02	6781.45	14.49	13.21
Dermaptera	9	2.43	14	2.76	342.58	0.73	1.97
Diptera	47	12.67	62	12.23	1161.08	2.48	9.13
Formicidae	26	7.01	38	7.5	5443.42	11.63	8.71
Gryllidae	6	1.62	9	1.78	274.07	0.59	1.33
Hymenoptera	17	4.58	25	4.93	2595.38	5.55	5.02
Insect larvae	35	9.43	47	9.27	6604.66	14.11	10.94
Isoptera	23	6.2	27	5.33	2439.13	5.21	5.58
Julidae	6	1.62	7	1.38	138.24	0.3	1.1
Lepidoptera	3	0.81	3	0.59	194.05	0.41	0.6
Neuroptera	7	1.89	8	1.58	819.68	1.75	1.74
Odonata	6	1.62	7	1.38	692.87	1.48	1.49
Ephemeroptera	2	0.54	2	0.39	277.89	0.59	0.51
Orthoptera	71	19.14	81	15.98	6282.69	13.42	16.18
Scorpiones	3	0.81	4	0.79	259.4	0.55	0.72
Trichoptera	9	2.43	12	2.37	3387.98	7.24	4.01
Plant materials	7	1.89	30	5.92	4923.87	10.52	6.11
Unidentified	41	11.05	55	10.85	3747.37	8.01	9.97
Total	371	100	507	100	46802.93	100	100

F—frequency of prey occurrence | N—number of prey items | V—prey volume (mm3) | IR—index of relative importance of each taxon, sampled in Hue City, Vietnam (n = 261 stomach contents).

males (15 prey species). The prey diversity index of adult females (2.6 with an evenness index of 0.7) was higher than that of adult males (2.36 with an evenness index of 0.66).

The number of prey items found in the stomachs of females was higher than that in males. The total prey volume was 28,490.85 mm^3 in females and 18,312.08 mm^3 in males. In females, prey measurements were as follows: length 9.28 ± 4.65 mm (F = 3.786, Sig. < 0.001), width 3.54 ± 2.4 mm (F = 2.501, Sig. = 0.001), and volume 98.69 ± 201.08 mm^3 (F = 1.655, Sig. = 0.048). In males, prey measurements were: length 8.5 ± 4.26 mm (F = 9.264, Sig. < 0.001), width 3.21 ± 1.7 mm (F = 8.451, Sig. < 0.001), and volume 84.04 ± 195.01 mm^3 (F = 3.040, Sig. < 0.001).

The type of prey differed significantly between males and females (F = 3.953, Sig. = 0.001). However, the relationship between prey and sex was not statistically significant (F = 1.395, Sig. = 0.221 > 0.05). In females, the composition, quantity, and volume of food items were all greater than in males, which is consistent with the efficiency of scale hypothesis (Forsman 1996) because females have larger body sizes and were able to consume larger prey items than males (Le et al. 2019). This evidence may partly explain the dietary differences of these prey groups between the sexes and why adult females consume a larger number of beetles, insect larvae, and termites (Figure 6), and the volume of food consumed by females is much larger than that of males.

Locations and season variation

We found that *Amolops spinapectoralis* is often confined to waterfalls and rarely ventures far from the water's edge. The species' diet reflects its limited range of activity and specific habitat. Since *Amolops spinapectoralis* is restricted to waterfall environments, its diet primarily consists of insects that inhabit these areas. For example, dragonflies, which rely on water for their reproductive cycle, were commonly found in the diet. On the other hand, we did not observe species like

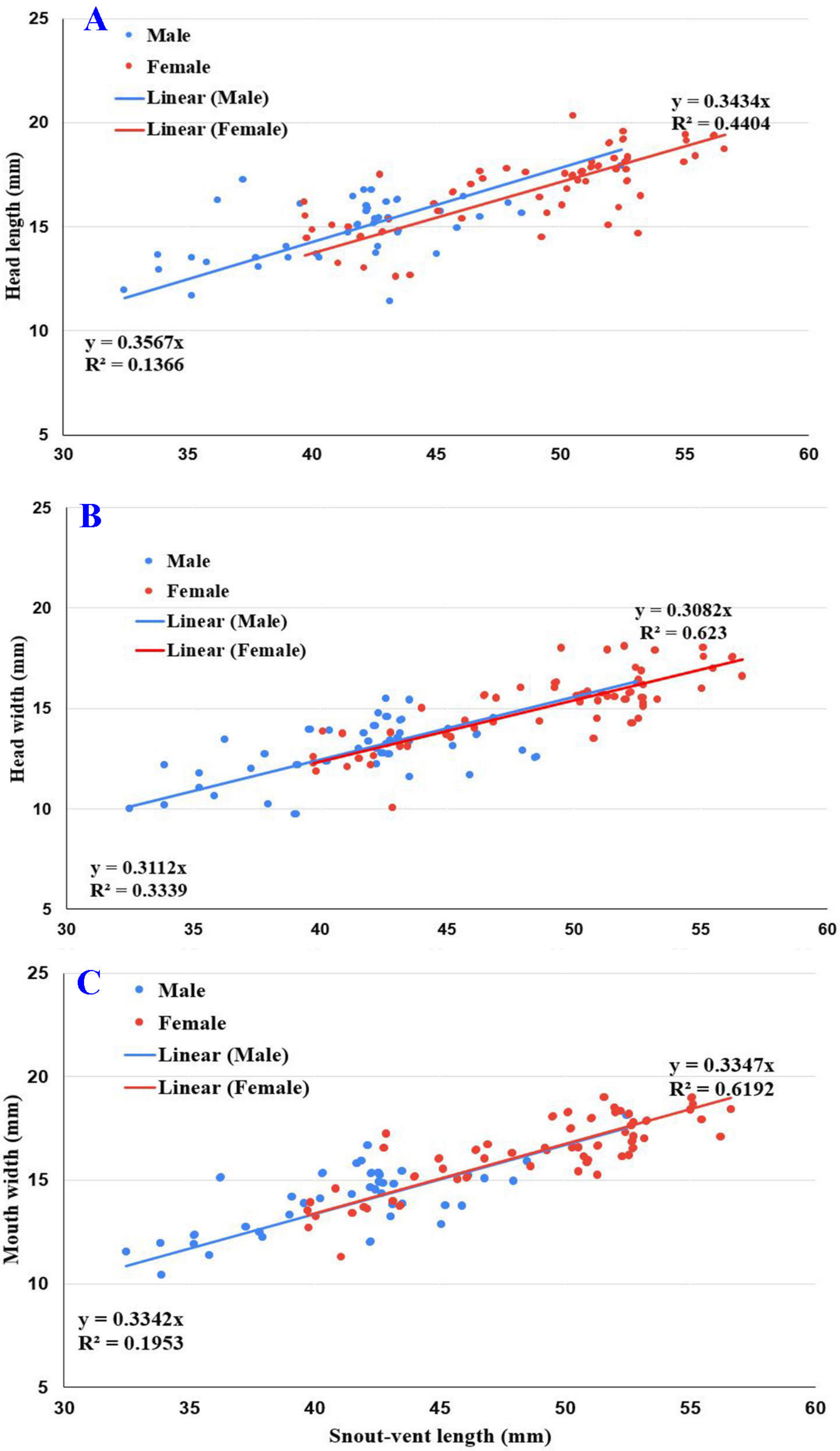

Figure 3. The relationships between: A—snout-vent length (SVL) and head length (HL) | B—head width (HW) | C—mouth width (MW) in adult males and females of *Amolops spinapectoralis*.

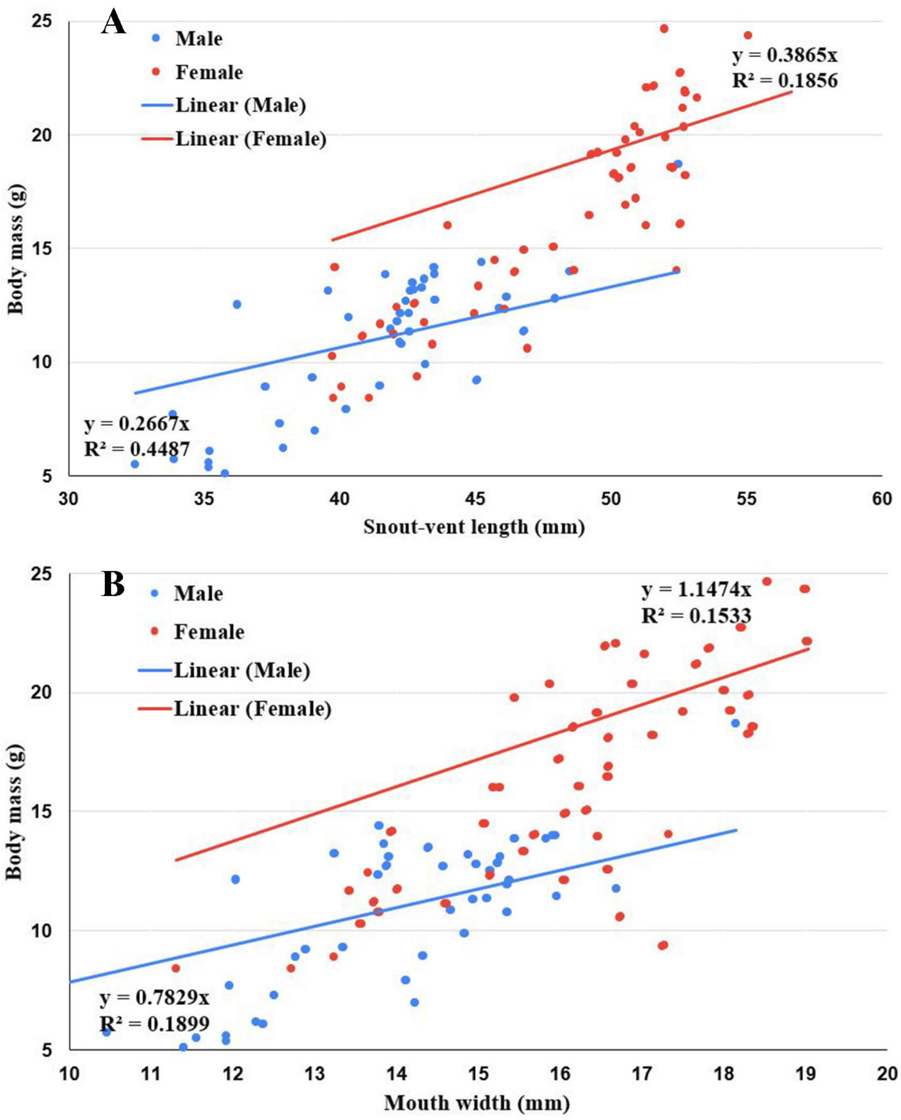

Figure 4. The relationships between: A—snout-vent length and body mass | B—mouth width and body mass of the *Amolops spinapectoralis*.

cockroaches and spiders in the food composition, as these organisms do not rely on water for reproduction and are rarely found in fast-flowing waterfall environments.

Seasonal prey consumption was recorded as follows: low-rainy season (204 items), auxiliary rainy season (153 items), and main rainy season (150 items) (F = 19.885, Sig. = 0.000). Prey varied between seasons, but this variation was not statistically significant (F = 1.338, Sig. = 0.223 > 0.05). In contrast, prey length and width did vary significantly between seasons (length: F = 13.454, Sig. < 0.001; width: F = 6.697, Sig. = 0.01).

Both the size and volume of prey consumed by both male and female *A. spinapectoralis* differed significantly between seasons (length: F = 13.454, Sig. < 0.001; width: F = 6.749, Sig. = 0.001; volume: F = 3.628, Sig. = 0.027).

Table 3. Simpson's index of diversity and Shannon-Wiener index among age groups, sites, and seasons in the diet of *Amolops spinapectoralis* from Hue City, Vietnam.

Contents	Shannon-Wiener index (Shannon-H, H')	Simpson's index 1/D
Adults	2.431	0.892
Subadults	2.383	0.883
Juveniles	2.163	0.869
Do Quyen (Phu Loc)	2.541	0.901
Thuong Lo (Khe Tre)	2.349	0.875
A Pat (A Luoi 4)	2.46	0.898
A little rainy season	2.515	0.903
Auxiliary rainy season	1.932	0.819
Main rainy season	2.214	0.872

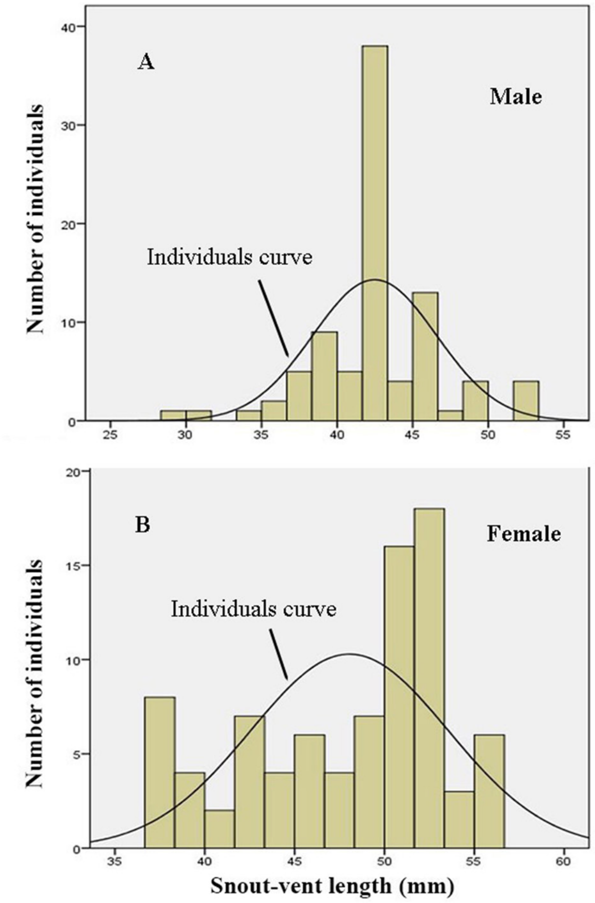

Figure 5. Normal distribution of snout-vent length (SVL, mm) for male (A) and female (B) *Amolops spinapectoralis* individuals, showing the distribution of individuals and sexual size dimorphism. For clarity, the upper bounds of the classes are shown. The curves in the figure are generated from a fit of the normal distribution for the data.

However, prey weight did not show significant seasonal variation (weight: F = 1.744, Sig. = 0.176). There were no significant differences in the size, volume, or weight of prey consumed across different localities (length: F = 0.24, Sig. = 0.815; width: F = 1.811, Sig. = 0.165; volume: F = 0.132, Sig. = 0.877; weight: F = 0.918, Sig. = 0.400).

In males, prey mass did not differ between seasons (F = 0.556, Sig. = 0.569), but prey size did (length: F = 8.355, Sig. < 0.001; width: F = 5.115, Sig. = 0.006). Prey mass and size consumed by males also did not vary significantly between localities. In females, prey mass did not differ by season (F = 1.744, Sig. = 0.176), but prey size did (length: F = 11.370, Sig. < 0.001; width: F = 597.496, Sig. < 0.001). Similar to males, prey mass and size consumed by females did not show significant differences across localities.

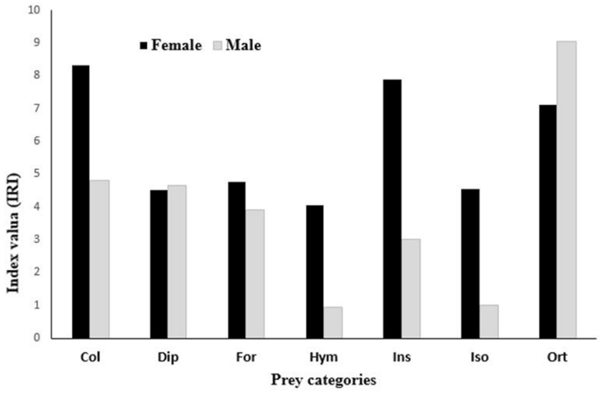

Figure 6. Index of relative importance (IRI) for major prey categories consumed by male and female *Amolops spinapectoralis* from Hue City, Vietnam: Col—Coleoptera | Dip—Diptera | For—Formicidae | Iso—Isoptera | Ins—Insect larvae | Iso—Isoptera | Ort—Orthoptera.

Discussion on seasonal factors

The seasonal prey numbers were: little rain (152 prey species), auxiliary rain (204 prey species), and main rain (151 prey species). Thus, we found that the secondary rainy season from May to August had a higher prey species than the other two seasons; the consistency of the data was shown in the main rainy season from September to December, when *A. spinapectoralis* had difficulty finding food in the high stream water conditions and stream frogs had to find shelter to avoid being swept away by the fast-flowing stream water. The low rainfall season is from January to April; however, January is still rainy, so foraging conditions remain difficult for *A. spinapectoralis*.

Morphometry and prey correlates

In the feeding ecology of this species, both SVL and MW were identified as important predictor variables for diet, as there were strong positive correlations between these morphological measurements (SVL and MW: r^2 = 0.4638, F = 147.737, Sig. < 0.001) and between MW and prey body mass (MW and prey BM: r^2 = 0.0534, F = 9.688, Sig. = 0.002).

In adult *A. spinapectoralis*, there was a significant positive correlation between mouth width (MW) and the size of prey consumed. However, regression analysis indicated that mouth width did not significantly affect prey size (mouth width and prey length: r^2 = 0.0024, F = 0.414, Sig. = 0.521; mouth width and prey width: r^2 = 0.0008, F = 0.135, Sig. = 0.714). Conversely, snout-vent length (SVL) was found to influence prey length (SVL and prey length: r^2 = 0.036, F = 6.437, Sig. = 0.012), but had no significant effect on prey width or mass.

Analyzing the relationship between prey size (length, width, and mass) and body size (SVL, MW) revealed that body size significantly influenced prey length ($r^2 = 0.064$, F = 5.792, Sig. = 0.004). However, body size did not have a significant effect on prey width ($r^2 = 0.0062$, F = 0.531, Sig. = 0.589) or prey mass ($r^2 = 0.0279$, F = 2.429, Sig. = 0.091).

In addition to habitat-driven prey availability, amphibians respond to various environmental factors. In our survey, we observed that during the auxiliary rainy season (from May to August) in the highlands, frequent fog significantly influences the composition and quantity of food, leading to emptier stomachs or stomachs containing only minimal or plant food. This results in a lower diversity and richness index of *A. spinapectoralis* diet during the auxiliary rainy season compared to both the little rainy season and the main rainy season (Table 3). A significant amount of plant material in the gut of frogs has been previously reported. This was reflected in a plant materials importance index (IRI) of 6.11%. These findings suggest that *A. spinapectoralis* has developed an adaptive response to the challenging conditions of its fast-flowing stream habitat (Image 1).

Environmental factor Influences

Environmental factors such as temperature, humidity, and rainfall affect the amount of prey consumed by Spinyback Torrent Frog. Analysis of the potential effects of temperature, relative humidity, and rainfall showed that these factors co-varied with season (overall: $r^2 = 0.6856$, F = 364.715, Sig. < 0.001). Temperature, relative humidity, and rainfall were significantly correlated with prey volume ($r^2 = 0.0219$, F = 3.734, Sig. = 0.011); however, prey mass was not significantly correlated with these climatic factors ($r^2 = 0.0128$, F = 2.175, Sig. = 0.09).

Multiple linear regression results indicated that temperature, relative humidity, and rainfall significantly affected prey numbers, with positive correlations overall ($r^2 = 0.0581$, F = 3.444, Sig. = 0.018). Specifically, temperature ($r^2 = 0.0234$, F = 4.070, Sig. = 0.045) and relative humidity ($r^2 = 0.0003$, F = 4.454, Sig. = 0.036) had significant positive effects, while rainfall showed an insignificant and negative correlation with prey numbers ($r^2 = 0.0005$, F = 0.085, Sig. = 0.771).

Distribution: This is the first record of *Amolops spinapectoralis* in Hue City with typical morphological characteristics as described above.

DISCUSSION

The body length, body mass, and head size of *Amolops spinapectoralis* in Hue City were different between the two sexes. Adult females had larger body length, head size, and body mass than males. These results are consistent with some data reported in many other rock frog species worldwide, such as *Amolops caelumnoctis* (Rao et al. 2007), *A. assamensis* (Sengupta et al. 2008), and *A. yunkaiensis* (Lyu et al. 2018). The morpholometric characteristics of our specimens agree well with the description by Inger et al. (1999). However, the special characteristic of *Amolops spinapectoralis* in Hue City is that the thoracic spines are not conical but obtuse (according to the description by Inger et al. 1999 of *A. spinapectoralis* in Gia Lai province, the thoracic stage is conical). With this morphological characteristic, many scientists confuse this species with *A. ricketti*. However, current data show that *A. ricketti* is not distributed in Vietnam. This confirms that *A. spinapectoralis* in Vietnam has some different morphological characteristics between regions.

In Vietnam, *A. spinapectoralis* has been recorded in the northern and central mountainous regions, including the provinces of Gia Lai, Da Nang, Quang Nam, Quang Ngai, Kon Tum, and Phu Yen (Frost 2025). Outside of Vietnam, it has been documented in Dakcheung District, Xekong Province, southeastern Laos, and is presumably found in adjacent northeastern Cambodia (Frost 2025).

To date, no dietary data have been reported for *A. spinapectoralis*, a poorly known frog species that was previously considered endemic to Vietnam (Nguyen et al. 2009). In this study, we identified 18 prey types in females and 15 prey types in males. Frogs are generally considered opportunistic carnivores, with their diet closely related to prey availability in their environment (Duellman & Trueb 1994). Their diet composition is primarily constrained by the availability and diversity of prey of suitable size (Wells 2007). Insects, the most frequent prey type, exhibit the highest species diversity, as previously documented by various studies. Insects are not only the most abundant prey in the environment but are also the most frequently consumed by frogs (Yousaf et al. 2010).

In general, the nutritional composition of *A. spinapectoralis*, which includes the suborder Orthoptera, termites, and insect larvae, may contain higher levels of protein (64.38~70.75%) and fat (18.55~22.8%) than those of Hymenoptera, Coleoptera, and Isoptera (according to Redford & Dorea 1984; Berenbaum 1996; Rumpold & Schlüter 2013). As insectivores, the natural diet of amphibians will consist of 30% to 60% protein (McWilliams 2008; Browne 2009).

Our comparison results indicate that *A. spinapectoralis* primarily engages in lying and waiting, consistent with the 'sit-and-wait' model. This result aligns with existing research on the activity and hunting habits of the Ranidae family (Hadfield et al. 2006).

The division of habitats for frog species to exploit food resources is quite clear. In the survey, we observed that *Quasipaa verrucospinosa* to also often appear in low-slope streams. In *A. spinapectoralis* diet, cockroaches and spiders are almost absent in the collected stomach contents. In the three research locations, we found that the abundance of beetles and grasshoppers in the habitats is consistent with the food composition in the stomach of this species.

Our finding aligns with observations of amphibians inhabiting fast-flowing waterfalls, where increased rainfall causes streams to rise, and flow velocity to increase, making it more challenging for the animals to seek shelter and forage. These results are consistent with studies on amphibians that highlight the influence of ecological and climatic pressures, while no significant role for sexual selection was identified (Pincheira-Donoso et al. 2020).

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