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Variation in dietary composition of granular spiny frogs (*Quasipaa verrucospinosa*) in central Vietnam

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The granular spiny frog, *Quasipaa verrucospinosa* (Bourret, 1937), is native to Vietnam and classified as Near Threatened due to environmental degradation, loss of forest and stream habitats and human exploitation. We collected stomach contents of *Q. verrucospinosa* using a nonlethal stomach-flushing technique from three stream sites in the rain forests of Thừa Thiên-Huế Province, central Vietnam, to investigate their food habits. Dietary analysis identified 2645 prey items of 27 orders and nine classes. Prey comprised mainly invertebrates, but also fishes, frogs and conspecific sub-adults. The major prey items as determined by frequency of occurrence, item count and percent volume were spiders, beetles, crabs, hymenopterans, grasshoppers, crickets and cicadas. Insects alone accounted for an importance value of 59.8%. The mean monthly prey volume consumed was positively and negatively correlated to temperature and rainfall, respectively. Consistent with the increased energetic needs prior to the main breeding season, the number of prey items and volume of prey consumed per frog were highest in the little rainy season. The volume of prey consumed was positively correlated with snout-vent length and mouth width of frogs, supporting the gape limitation hypothesis. Despite their larger size, however, females did not consume greater numbers of prey items or larger-sized prey than males. Adults consumed a higher diversity of prey and higher proportions of Araneae and Hemiptera than sub-adults, whereas females had a more even diet than males and consumed a higher proportion of Orthoptera.

Key words: Anura, diet, feeding, frogs, stomach content, *Quasipaa verrucospinosa*, Vietnam

INTRODUCTION

Animal food habits offer essential information for constructing food webs (Elton, 2001). Dietary variation may provide additional insights for our understanding of life history features, resource and habitat use, population dynamics and interspecific interactions of species (e.g., Toft, 1980). In terrestrial and freshwater habitats, anurans are predators of many invertebrates and sometimes even vertebrates, and play a crucial role in energy and nutrient cycling between these two systems (Duellman & Trueb, 1994; Wells, 2007). However, detailed information on ecological needs of many anurans is either insufficient or lacking. Anurans are among the most threatened terrestrial vertebrates (Stuart et al., 2004; Alford, 2011), and population declines are likely to result in a particularly high diversity loss in tropical regions, where vast numbers of species reside and often exhibit high endemism (e.g., Fauth et al., 1989; Allmon, 1991; Vitt & Caldwell, 1994).

Anurans are usually considered as opportunistically foraging generalist predators (Wells, 2007). This intuitively predicts that more aquatic anurans consume larger numbers of aquatic prey than do semiaquatic or terrestrial species (Hothem et al., 2009; Rosa et al., 2011). It also generally helps to explain why anurans living in tropical-subtropical regions have more diverse diets than temperate species (e.g., Moseley et al., 2005; Ugarte et al., 2007; Hothem et al., 2009; Brito et al., 2013). Nevertheless, anuran diets that specialise on specific prey types such as Acari, ants, beetles or termites are also reported from the tropics (Isacch & Barg, 2002; Bonansea & Vaira, 2007; Valderrama-Vernaza et al., 2009; Brito et al., 2013; Rodrigues & Santos-Costa, 2014), and may be associated with foraging mode, season and habitat (Toft, 1980).

The granular spiny frog, *Quasipaa verrucospinosa* (Bourret, 1937) is a dicroglossid frog native to Vietnam (Frost, 2014). All 12 species of the genus *Quasipaa* are narrowly distributed from northeastern Indochina to southern and southwestern China (Nguyen et al., 2009; Frost, 2014), and are classified as either at least Near Threatened or as Data Deficient (IUCN, 2013). In Vietnam, *Q. verrucospinosa* resides only in headwaters of a few montane streams in primary forests in the western and southern Thừa Thiên-Huế Province (Ngo & Ngo, 2011), where intact rainforest habitats remain and represent a recognised biodiversity hotspot rich in

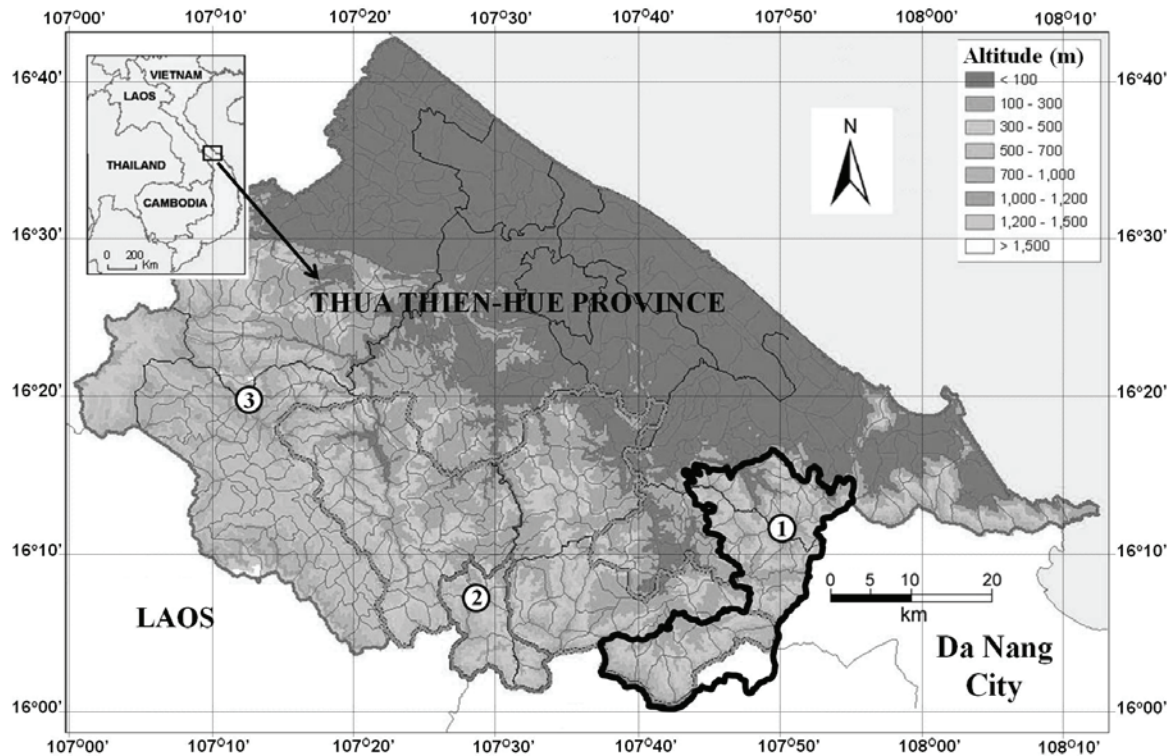*Correspondence: Ya-Fu Lee (yafulee@mail.ncku.edu.tw)*

Fig. 1. Map of Thừa Thiên-Huế Province showing the three localities: (1) Mang Stream, Bach Ma National Park; (2) Ba Rang Stream, Sao La Conservation Area; and (3) Dong Ngai Stream, Phong Dien Nature Reserve (A Luoi District), where granular spiny frogs were collected.

endemic species (Myers et al., 2000). In many parts of this region, however, frog populations have been declining over recent decades due to over-exploitation and other large-scale disturbances such as degradation of forested habitats and stream sedimentation from unselective or destructive logging (Ngo & Ngo, 2009; Ngo et al., 2009).

This study presents the first comprehensive investigation on the dietary composition and spatiotemporal variation of granular spiny frogs across its range in central Vietnam. We also compared the diets of frogs of different sexes and age groups to examine sex-dependent dietary variation, and tested the hypothesis that the size of prey consumed and the diet breadth are correlated with morphometry. Our study is the first to focus on dietary variation and its ecological correlates for one of the least-studied dicroglossid frogs in southeast Asia, providing baseline information for understanding resource use patterns of the genus *Quasipaa*.

MATERIALS AND METHODS

Study sites
Field work took place in Thừa Thiên-Huế Province, Vietnam (15°59'30"~16°44'30"N, 107°00'56"~108° 12'57"E). This area is characterised by tropical climate, with annual temperature averaging 24.4±0.41°C (ranging from 15.8±0.52°C in January to 29.7±0.71°C in June) and an annual mean precipitation of 4980±377 mm. A relatively dry period extends from January to April, with monthly rainfall of around 120 mm (mean: 121±19 mm). Most rainfall is concentrated in the monsoon season (September to December, monthly mean of 738±96 mm) and the little rainy season from May to August (monthly

mean of 173±23 mm; Nguyen et al., 2004). The study area is dominated by montane rainforests at elevations of 700~1400 m and cloud forests from above 1400 m up to the summit at 1774 m (Nguyen et al., 2004).

Sample collections
We captured frogs from three localities at roughly the same elevations: Mang Stream (1455 m) in Bach Ma National Park, Ba Rang Stream (1546 m) in the Sao La Conservation Area and Dong Ngai Stream (1579 m) in the A Luoi District (Fig. 1). During two nights in each month from January 2008 to December 2009, a team of six people conducted nocturnal surveys from 2000 to 0200 hours in each of the three streams. We visually searched for frogs using spotlights, aided by their calls, in water and up to 15 m away from the stream over a length of 3 km, collecting frogs by hand. Sex was determined by the number of spinules on back warts, the presence or absence of pectoral spines, hypertrophied forearms, callosities on finger I of the forelegs and a distinct tympanum. Sub-adults were distinguished from adults by snout-vent length (SVL) and body colour, with reddish brown characterising sub-adults and chrome yellow characterising adults (Ngo & Ngo, 2011). We measured mouth width (MW) and SVL with a digital caliper and body mass (BM) with an electronic balance. We used visible implant elastomer tags (Nauwelaerts et al., 2000; Hoffmann et al., 2008) to mark each frog.

We placed frogs individually in labelled bags for stomach contents collection within 30 min of capture (Caldwell, 1996). We adopted stomach-flushing (Griffiths 1986; Leclerc & Courtois, 1993) to obtain stomach contents without sacrificing frogs. We used different

Table 1. Dietary composition (%) of granular spiny frogs (*Quasipaa verrucospinosa*) with regard to frequency of occurrence (*F*), number of items (*N*), and volume (*V*), and the overall importance value (*I*) of each prey (*n*=539 frogs considered).

Prey	Frequency (F)	Number (N)	Volume (V)	Importance (I)
Arachnida (Araneae)	13	21.5	15.2	16.57
Insecta				
Blattodea	2.8	2.1	3.4	2.76
Coleoptera	12.9	18.7	17.6	16.39
Collembola	0.3	0.3	0.1	0.21
Dermaptera	4.3	3.1	2.5	3.32
Diptera	4.4	3.4	3.7	3.81
Ephemeroptera	0.2	0.2	0.3	0.24
Hemiptera	5.4	4	7.7	5.71
Hymenoptera (Formicidae)	7.8	5.9	0.7	4.83
Hymenoptera (others)	9.9	7.2	1	6.01
Isoptera	0.3	0.2	0.1	0.18
Lepidoptera	2.5	1.9	4.7	3.04
Neuroptera	0.2	0.2	0.2	0.2
Odonata	0.4	0.3	0.7	0.46
Orthoptera	12.2	9.6	6.6	9.45
Phasmatodea	0.5	0.5	1.6	0.86
Insecta larvae	2.6	2.1	2.3	2.34
Diplopoda	1.8	1.4	2.2	1.81
Chilopoda	0.4	0.3	0.7	0.49
Crustacea				
Decapoda	13.3	13.6	20.7	15.86
Isopoda	1.7	1.3	0.7	1.24
Clitellata				
Lumbriculida	0.3	0.3	0.3	0.3
Megadrili	0.1	0.04	0.04	0.04
Gastropoda (Mesogastropoda)	0.9	0.7	1.7	1.09
Osteichthyes				
Cypriniformes	0.2	0.1	1.4	0.56
Perciformes	0.5	0.3	1.6	0.78
Amphibia (Anura)	0.3	0.2	1.6	0.73
Unidentified	0.8	0.6	0.7	0.73

sizes of soft catheter tubes (2, 3 or 5 mm inner diameter) with appropriately-sized syringes and different amounts of clean water (60, 120 or 180 ml) for frogs of different SVL (≤60 mm, >60~≤100 mm and >100 mm). Each frog was stomach-flushed only once following the guidelines approved by the American Society of Ichthyologists and Herpetologists for animal care (Beaupre et al., 2004). After flushing, frogs were monitored for vigour and body conditions and released within 90 min at the place of capture. Samples were preserved in 95% ethanol for later analyses, and housed along with seven voucher specimens at the Faculty of Biology, Hue University.

Stomach content analysis

We sorted and identified prey items in each stomach sample using a dissecting microscope (MSL4000-20/40-IL-TL Standard Stereo Microscope, 10×2 magnification) and reference keys (Thai, 2001; Triplehorn & Johnson, 2005). Prey items were identified to the lowest possible taxonomic level; in a few cases nearly intact prey allowed for the identification to species level. We measured the length from head to thorax and the width at the widest section of the body of each prey item identified with a digital caliper to the nearest 0.01 mm. Materials such as sand, stones and vegetation were excluded from analyses, assuming an accidental ingestion (Parker & Goldstein, 2004; Hothem et al., 2009).

We determined the frequency of occurrence (*F*) and the percent count (*N*) for each prey item identified. Both parameters were standardised, ranging from 0 to 100%, and assumed to represent the relative commonness of each prey item in the frog's diet. The frequency of occurrence was calculated as the number of stomach samples in which a particular prey was identified, divided

Table 2. Dietary composition (%) of granular spiny frogs (*Quasipaa verrucospinosa*), and overall diet breadth (Levin's measure) at three different study sites. Sample sizes are in parentheses. [1]Including Phasmatodea; [2]including Collembola, Ephemeroptera, Isoptera, Odonata, and Neuroptera; [3]Decapoda and Isopoda; [4]Lumbriculida and Megadrili; [5]Cypriniformes and Perciformes.

Prey	Mang (177)	Ba Rang (183)	Dong Ngai (179)
Arachnida (Araneae)	23.2	19.8	22
Blattodea	1.6	2.5	2.2
Coleoptera	19.9	17.9	18.5
Collembola	0.4	–	0.5
Dermaptera	3.3	4.7	1.1
Diptera	2.3	4.2	3.3
Hemiptera	2.9	4.6	4.2
Hymenoptera	15.3	10.2	14.6
Lepidoptera	2.5	1	2.5
Orthoptera[1]	7.7	11.8	10.3
Other insects[2]	2.2	0.9	0.7
Insect larvae	2.2	2.1	1.9
Diplopoda	0.7	2.1	1.3
Chilopoda	0.3	0.5	0.2
Crustacea[3]	15.1	14.1	15.5
Clitellata[4]	0.8	–	–
Gastropoda (Mesogastropoda)	0.7	0.7	0.7
Osteichthyes[5]	0.3	0.8	0.1
Amphibia (Anura)	–	0.4	0.2
# Category (diet breadth)	21 (6.64)	24 (9.75)	21 (7.45)

by the sum of the numbers of samples that contained each identified prey (Lee & McCracken, 2005). The percent count of a prey item at each site or sampling unit (date or month) was averaged over all samples examined for a site or a sampling unit to provide an index of the proportional contribution of each prey item to the diets of the frogs sampled. We estimated the volume of each prey item by the formula for a prolate spheroid, where volume=$(4\pi/3) \times (L/2) \times (W/2)^2$ (Caldwell & Vitt, 1999; Magnusson et al., 2003), and calculated the relative percent volume (*V*) among prey items. *L* and *W* each represented the respective length and width of a prey item identified in a sample, which came from actual measurements whenever possible or a best estimate. We used the importance index, *I*, to determine the overall importance of each prey taxon in the diet (Biavati et al., 2004), where $I=(F+N+V)/3$. We used Levin's measure, $B=1/\Sigma p_j^2$, to estimate diet breadth, where p_j is the fraction of prey item *j* in the diet. We adopted the reciprocal Simpson's heterogeneity index, $1/D$, to calculate dietary heterogeneity, where $D=\Sigma([n_i(n_i-1)]/[N(N-1)])$, n_i is the number of prey items in the ith prey category and *N* is the total number of prey categories (Krebs, 1999). The 95% confidence intervals (CI) of the index were assessed by $(\Sigma\Phi_i/n) \pm t_{0.05(n-1)}SE_\Phi$ using the jackknife method, where $\Phi_i=nSt-(n-1)St_{-i}$, $SE_\Phi=\sqrt{[\Sigma(\Phi_i-(\Sigma\Phi_i/n))^2/n(n-1)]}$, *St* is the sample statistic of $1/D$, St_{-i} is the sample statistic as calculated missing out each sample *i* in turn, and *n* is the number of samples (Magurran, 2004).

Data analysis

We performed statistical analyses using STATISTICA v.10 (StatSoft, Tulsa, OK, USA) for Windows 2000, and set $p<0.05$ as the significance level. Data are presented as mean±standard error (SE) unless otherwise noted and proportional data were arcsine-transformed to meet the normality requirement (Zar, 2010). We used chi-square statistics to examine the distribution of stomach content samples from frogs of different sex/age groups among seasons and sites. Multiple regression analysis was used to examine the relationships between body size and prey size, and between climatic factors and volumes of different prey items. We adopted multivariate analysis of variance (MANOVA) and subsequent Fisher's least significant difference (LSD) as post-hoc comparisons to test for differences in body size (BM, MW and SVL) and prey size variables (length, width and volume) among frogs. We also used MANOVA to examine the effects of year, season and site on the variance in percent volume of each prey in the diet of frogs (Zar, 2010). Prey items with a frequency of occurrence below 0.5% were considered rarely encountered and excluded from the latter analysis.

RESULTS

Frog captures and morphometric differences

We caught 539 frogs from the three stream sites, comprising 245 males, 205 females and 89 sub-adults. Sample composition did not deviate from a random distribution among sites ($\chi^2=0.517$, df=4, $p>0.9$) or
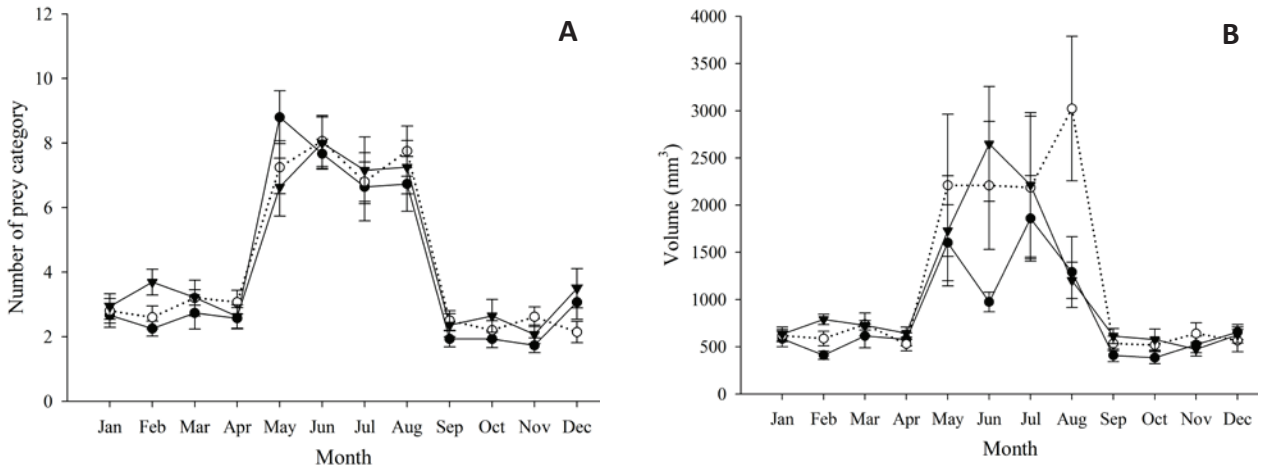
Fig. 2. Mean (±SE) monthly (A) number of prey categories and (B) total volume consumed by granular spiny frogs (*Quasipaa verrucospinosa*) in Mang Stream (–●–), Ba Rang Stream (···○···), and Dong Ngai Stream (–▼–).

seasons (χ^2=0.184, df=4, p>0.9). Frog morphometry was not affected by sampling site (Wilks' λ=0.984, $F_{6, 1020}$=1.42, p=0.21) but differed among sex and age groups (Wilks' λ=0.139, $F_{6, 1020}$=314.94, p<0.001). Females had larger mouth widths (MW: 28.14±0.40 mm) and snout-vent lengths (SVL: 108.22±1.54 mm) and were heavier (BM: 134.68±4.49 g) than adult males (MW: 24.95±0.25 mm, SVL: 96.78±1.02 mm; BM: 89.27±2.33 g; LSD, all p values <0.001). Adults were larger and heavier than sub-adults (MW:12.07±0.24 mm, SVL: 36.30±0.56 mm, BM: 4.91±0.33 g; LSD, all p values <0.001). These parameters

Table 3. Dietary variation of granular spiny frogs (*Quasipaa verrucospinosa*) across seasons (1: dry season; 2: little rainy season; 3: main rainy season) and among stream sites (MA, Mang Stream; BR, Ba Rang Stream; DN, Dong Ngai Stream). LSD, *p<0.05, **p<0.01, ***p<0.001.

Prey	Season	Site	Season × Site
Araneae	2>1***		
	2>3***		
Blattodea	2>1*		
	2>3**		
Coleoptera			DN > MA at 1**
			DN > BR at 3*
			2 > 1 at DN*
Decapoda			DN > MA at 1***
			DN > BR at 2***
			DN > BR at 3***
			2 > 3 at DN***
Dermaptera		DN > MA**	
		DN > BR**	
Diptera		DN > MA**	
Hemiptera	2 > 3**	DN > MA**	
		DN > BR**	
Lepidoptera		DN > MA**	
		DN > BR**	
Orthoptera	2 > 1*		
	2 > 3**		

were also positively correlated with each other (SVL-MW: r=0.96, $F_{2, 537}$=6407.24, p<0.001; MW-BM: r=0.94, $F_{2, 537}$=3806.72, p<0.001; SVL-BM: r=0.98, $F_{2, 537}$=16833.58, p<0.001).

Dietary composition

We counted 2645 identifiable food items comprising 27 prey taxa from 25 orders, nine classes and four phyla (Arthropoda, Annelida, Chordata and Mollusca). Vertebrate prey encompassed the cyprinid fish *Onychostoma laticeps* Günther, 1896, the goby *Rhinogobius duospilus* (Herre, 1935), green cascade frogs *Odorrana chloronota* (Günther, 1876) and sub-adult *Q. verrucospinosa*. Insects dominated the diet, accounting for 66.7% in frequency of occurrence, 59.7% in prey items and 53.2% of the total volume, generating an overall importance value of 59.8% (Table 1). We found no recognisable contents in 21 samples (5 females and 16 sub-adults; 3.9% of the captures).

The major prey orders by rank, defined as an importance value >5%, and the respectively identified species included Araneae [*Latouchia cunicularia* (Simon, 1886); *Myrmarachne manducator* (Westwood, 1841); *Nephila maculata* (Fabricius, 1793)], Coleoptera [*Adoretus compressus* (Weber, 1801); *Cicindela sexpunctata* Fabricius, 1775], Decapoda [*Ovitamon artifrons* (Buerger, 1894); *Thelphusula baramensis* (De Man, 1902)], Hymenoptera [*Cladius pectinicornis* Geoffroy, 1785); *Oecophylla smaragdina* (Fabricius, 1775)], Orthoptera [*Gryllotalpa brachyptera* Tindale, 1928; *Oxya chinensis* (Thunberg, 1815)] and Hemiptera [*Platypleura kaempferi* (Fabricius, 1794); *P. nigrosignata* Distant, 1913]. They were followed by Diptera, Dermaptera and Lepidoptera. These prey taxa collectively accounted for nearly 85% of the importance value but varied in their respective contributions. Hymenoptera was the most frequently found and Araneae the most numerically abundant, whereas Decapoda was the most voluminous and Hymenoptera the least voluminous prey (Table 1).

Dietary variation

We found no between-year differences (Wilks' λ=0.871, $F_{15, 22}$=0.22, p=0.998) in the volumes of major prey
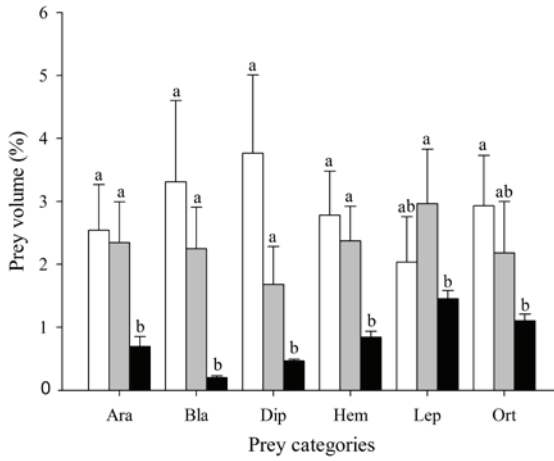
Fig. 3. Mean (±SE) percent volumes of prey items in the diets of female, male and sub-adult granular spiny frogs (*Quasipaa verrucospinosa*). Ara: Araneae; Bla: Blattodea; Dip: Diplopoda; Hem: Hemiptera; Lep: Lepidoptera; Ort: Orthoptera. Different letters above the three frog groups for the same prey indicate a significant difference in proportional volumes. Anura, Collembola, Cypriniformes, Ephemeroptera, Isoptera, Lumbricidae, Megadrili, Neuroptera, Odonata, Perciformes, Phasmatodea, and Scolopendromorpha were excluded from the analysis due to low frequencies of occurrence (<0.5%).

present, and samples were pooled for further analyses. Diet varied both among sites (Wilks' λ=0.018, $F_{30, 44}$=5.55, p<0.001) and among seasons (Wilks' λ=0.016, $F_{30, 44}$=5.99, p<0.001). The spatial variation was evident when the diets were compared among the three stream sites, showing a general similarity among streams but a broader diet at Ba Rang Stream (Table 2). The frogs sampled at Dong Ngai Stream consumed higher proportions of Dermaptera, Diptera, Hemiptera and Lepidoptera than those from the other two sites (Table 3), whereas the frogs at Ba Rang Stream had a more diverse (Simpson heterogeneity index, SH=8.71, CI: 5.70~11.72) and more even diet (Simpson's evenness index, SE=3.01) than samples from Mang Stream (SH=7.30, CI: 5.26~9.33, SE=2.04) and Dong Ngai Stream (SH=7.96, CI: 6.67~9.25, SE=1.286).

The mean monthly prey volume consumed by *Q. verrucospinosa* was positively correlated with temperature (r=0.338, $F_{2, 69}$=9.03, p=0.004) and negatively correlated with rainfall (r=-0.619; $F_{2, 69}$=43.46, p<0.001). The mean number of prey items (9.6±0.31; $F_{2,515}$=349.671, p<0.001) and volume of prey (1944.64±171.95 mm³; $F_{2,515}$=61.561, p<0.001) consumed per frog were higher in the little rainy season than in the dry season (3.2±0.13; 618.20±23.36 mm³) and the main rainy season (2.6±0.13; 540.47±24.88 mm³; Fig. 2). Diets during the little rainy season contained higher proportions of Araneae, Blattodea, Hemiptera and Orthoptera. Dietary variation, however, also revealed season-site interactions (Wilks' λ=0.007, $F_{60, 88}$=2.25, p<0.005), where differences in the proportions of Coleoptera and Decapoda occurred between paired comparisons of seasons at specific sites, or vice versa (Table 3).

A stomach sample on average contained 4.91±1.59 prey items (range: 0~7). Adult males (25 prey categories; SH=11.68, CI: 10.40~12.95) and females (23 prey categories; SH=10.00, CI: 7.06~12.94) consumed more diverse prey than sub-adults (14 prey categories; SH=10.72, CI: 9.43~12.01) with higher individual variation in females, whereas females had a more even diet (SE= 2.94) than males (SE=1.28) and sub-adults (SE=1.29). Among different sex/age groups (Wilks' λ=0.202, $F_{15,37}$=9.75, p<0.001), adult females and males both fed on higher proportions of Araneae, Blattodea, Diplopoda and Hemiptera (LSD, p values <0.05 or <0.01), whereas females additionally consumed a higher proportion of Orthoptera (LSD, p<0.05) and males took a higher proportion of Lepidoptera (LSD, p<0.05) than sub-adults (Fig. 3). Prey size averaged 15.47±5.64 mm in length (range: 2.19~57.32), 4.33±2.01 mm in width (range: 1.01~24.35), and 218.37±462.41 mm³ in volume (range: 1.17~10,229.31). Males and females both consumed larger prey ($F_{2, 2644}$=14.80, p<0.0001; male: 15.60±6.04 mm; female: 15.82±5.30 mm) and greater volumes ($F_{2, 2644}$=6.67, p=0.001; male: 233.33±544.81 mm³; female: 231.69±454.30 mm³) than sub-adults (length: 14.11±5.36 mm; volume: 142.20±130.82 mm³). The volume of prey consumed was positively correlated with SVL (r=0.30, $F_{2, 516}$=49.64, p<0.001) and mouth width (r=0.31, $F_{2, 516}$=53.49, p<0.001; Fig. 4).
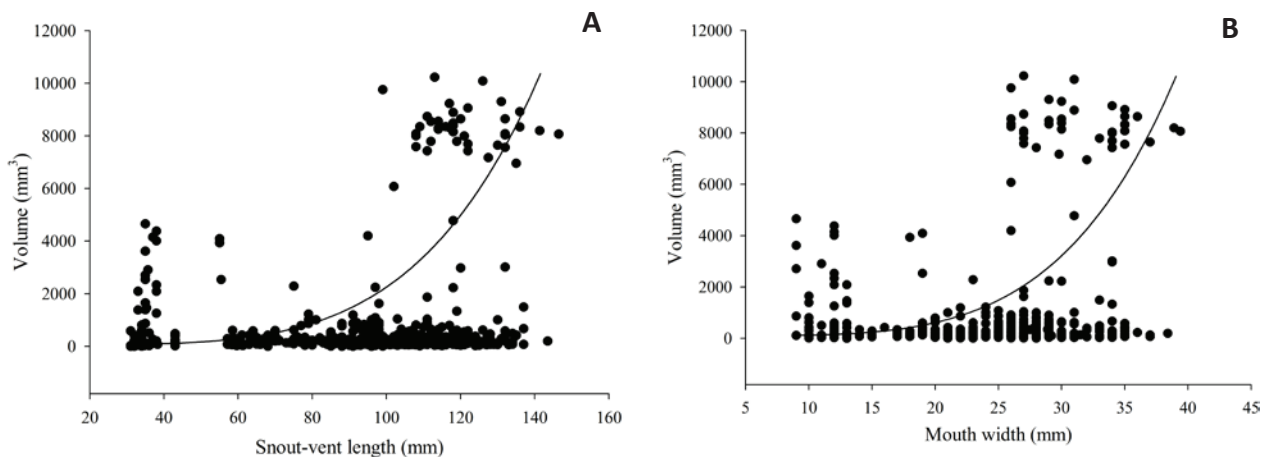

Fig. 4. Correlations between the volume of prey consumed and (A) snout-vent length, and (B) mouth width, of granular spiny frogs (*Quasipaa verrucospinosa*).

DISCUSSION

Our study documents one of the most diverse diets for tropical anurans ever recorded (e.g., Stewart & Woolbright, 1996; Biavati et al., 2004; Wachlevski et al., 2008; Leavitt & Fitzgerald, 2009; Quiroga et al., 2009; Caldart et al., 2012; Brito et al., 2013). Amphibians are generally opportunistic foragers, and their dietary composition is largely constrained by the availability and diversity of appropriately sized prey (Wells, 2007). It is not surprising that tropical-subtropical anurans have often been reported feeding on spiders and insects, particularly beetles and ants (e.g., Stewart & Woolbright, 1996; Biavati et al., 2004; Leavitt & Fitzgerald, 2009; Quiroga et al., 2009; Caldart et al., 2012; Brito et al., 2013).

Major prey taxa such as spiders, hymenopterans (including ants) and crabs differed in their respective contributions in terms of frequency of occurrence, count and volume. Spiders and ants are prominent components of the leaf-litter fauna of many tropical rainforests (e.g., Brito et al., 2012). Ants in particular often outnumber other prey, are easy for frogs to catch and are energetically rewarding (Redford & Dorea, 1984; Marconi et al., 2002). The disproportionally lower percent volume of ants compared to their frequency of occurrence and percent count was presumably due to their much smaller body size and less sclerotised exoskeleton compared to crabs and cicadas (ca. 10~25 mm in length and 4~13 mm in width; B.V. Ngo unpublished data).

The diet of *Q. verrucospinosa* comprised of aquatic as well as ground and arboreal terrestrial prey, which is in line with their general habitat use (Ngo & Ngo, 2009). The major prey items appeared to be common among all the populations studied. Yet, we noted dietary variation among sites, with the broadest diet found for the Ba Rang Stream. The Ba Rang Stream is situated in the preserved Sao La Conservation Area containing intact primary forests, while the other two streams have suffered different extents of disturbance (logging and transportation development). Dietary variation is also evident when compared to that analysed previously for a population sampled from A Pat Stream in the A Luoi District (Ngo et al., 2009), which contains a lower proportion of Araneae but higher proportions of Orthoptera, Crustacea and Clitellata, and an overall broader diet. The dietary discrepancies among sites and between our and previous studies might largely reflect the local availability of prey (Burton, 1976; Ortega et al., 2009). Although we did not assess prey availability, the relative importance of the major prey items corresponds to their general patterns of abundance (Ngo et al., 2009).

The volume and number of prey items taken by *Q. verrucospinosa* also showed seasonal variation. Prey volume was positively correlated with temperature and negatively correlated with precipitation. Animals typically increase their demands, intake and storage of energy and nutrition during or prior to life-cycle events such as migration, hibernation (Harvey & Weatherhead, 2006; Strain et al., 2012), and, most notably, reproduction (for anurans, see Jørgensen et al., 1986; Duellman & Trueb,

1994; Wells, 2007). Higher food intakes were attained in the little rainy season, coinciding with the prime breeding season of *Q. verrucospinosa* (Ngo et al., 2013), whereas the lowest prey volume occurred in the main rainy season during winter.

We found positive correlations between prey size and body size, which supports our prediction and is consistent with general findings for anurans (Wells, 2007; but see Quiroga et al., 2009). Larsen (1992) indicated that under *ad libitum* conditions the sizes of prey ingested are correlated to stomach capacity of a frog and its urge to feed, which is largely conditioned by daily and annual rhythms such as energy demands. Perhaps due to the limited gape size and the lower body capacity, sub-adults have a limited ability to consume large prey. Adult *Q. verrucospinosa* also consumed a broader range of prey than sub-adults. Previous studies indicated that body size, mouth width and the availability of prey play a decisive role in determining the size of prey and diet composition for amphibians (Ortega et al., 2009). Sub-adults occurred in each of the three localities along with adult frogs throughout the year, and so presumably were exposed to the same prey. The differences in diet between adults and sub-adults may reflect foraging activity associated with microhabitats. Adults occurred in both terrestrial and aquatic habitats containing diverse features like cascades, waterfalls, large tree hollows, rock crevices, caves and a broader range of humidity and temperature, whereas sub-adults often forage near water and in areas with flatter terrain, secondary forests and low prey availability (Ngo & Ngo, 2009; B.V. Ngo unpublished data).

There is currently no evidence that males and females have different foraging habitats or activity times (Ngo et al., 2013). That prey size and diet volume increased with frog's body size is consistent with the gape limitation hypothesis (Werner et al., 1995). Despite their larger size, however, females did not consume greater numbers of prey items or larger-sized prey, contradicting the size-efficiency hypothesis (Brooks & Dodson, 1965; Forsman, 1996). Remarkably, females consumed higher proportions of grasshoppers and crickets whereas males consumed a higher amount of moths such as *Scirpophaga incertulas* (Walker, 1863). Egg production is costly, and the fecundity and egg size of amphibians often depend upon the nutritional quality of food (Wilbur et al., 1974; Ferreira et al., 2012). Prior to the breeding season, female *Q. verrucospinosa* feed more and fat bodies increase (Ngo et al., 2013). In general, orthopterans are richer in protein (64.38~70.75%) and fat contents (18.55~22.8%) than lepidopterans (proteins: 48.7~58%, fat: 5.25~14.3%; Rumpold & Schlüter, 2013). Further studies on microhabitat use and foraging tactics of females and males are needed to discern between food availability and needs for both sexes.

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