



Notes on the diets of five amphibian species from southwestern Taiwan

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The stomach contents of 156 anuran specimens, of five species, from the families, Bufonidae, Dicroglossidae and Microhylidae, that had been accidentally collected by pitfall traps, were examined to contribute to the understanding of the diets of these anuran species. Three thousand four-hundred and six prey items, from 21 orders of 6 classes were recorded, and ants (Formicidae) were the most numerous prey items in the diets of all five anuran species. *Fejervarya limnocharis* had the broadest dietary niche breadth, followed by *Duttaphrynus melanostictus*, *Microhyla fissipes*, *Micryletta stejnegeri*, and *Microhyla heymonsi*, in that order. There were also substantial dietary overlaps among the anurans described herein, and the degree of overlap ranged from ca. 80% to ca. 95%. The results of this study provides further support for the suggestion that *Duttaphrynus melanostictus* and *Fejervarya limnocharis* are dietary generalists that opportunistically prey on whatever prey of suitable size is present in any given habitat. As for the microhylids, the results of this study support the conclusion that members of the genus *Microhyla* are ant-specialists. The results of this study also suggests that all five species are predators of primarily terrestrial arthropods. Future dietary niche partitioning and competition studies should attempt to identify the major prey categories to the species, or at least genus levels, because there may be variations in the species, even of the same order, in the diets of anurans.

INTRODUCTION

Anurans are important parts of most terrestrial and freshwater ecosystems in many parts of the world (Wager, 1986; Channing, 2001; Whiles *et al.*, 2006). Anurans are often a food source for a variety of invertebrates and vertebrates (Wager, 1986; Channing, 2001; Whiles *et al.*, 2006; Toledo *et al.*, 2007). Their larvae are also important primary consumers in wetlands, and often compete with other aquatic herbivores, such as mosquito larvae (Wager, 1986; Channing, 2001; Mokany & Shine, 2003a; Mokany & Shine, 2003b; Altig *et al.*, 2007). In addition, as adults, and occasionally as larvae, anurans are predators of mainly arthropods and other invertebrates (Wager, 1986; Channing, 2001; Whiles *et al.*, 2006).

An understanding of the natural history and field ecology of amphibians and other herpetofauna are essential for successful conservation and management programs (Bury, 2006). However, amphibian populations worldwide are in decline (Wake, 1991; Gardner, 2001; Blaustein & Kiesecker, 2002). There is thus a need to expand the understanding of the natural history of amphibians and their ecological roles in a manner that would not exert additional pressures on existing populations. As has been suggested for taxonomical studies (Mittermeier & Carr, 1994), the use of specimens in collections in institutions, such as museums, or any other existing collected specimens, can be instrumental in this regard.

A crucial part of the natural history of an animal is its diet, because not only does it reveal the source of the animal's energy for growth, maintenance, and/or reproduction (Dunham *et al.*, 1989; Zug *et al.*, 2001), but it also indicates part of the ecological roles of the animal. Since there may be temporal and spatial variations in the diet of a species (e.g. Mahan & Johnson, 2007; Araújo *et al.*, 2009; Quiroga *et al.*, 2011), there is a need for dietary descriptions from different localities. Herein, we contribute to the understanding of the diet of five anuran species from southwestern Taiwan, by describing the stomach contents of specimens that had been accidentally collected by pitfall traps.

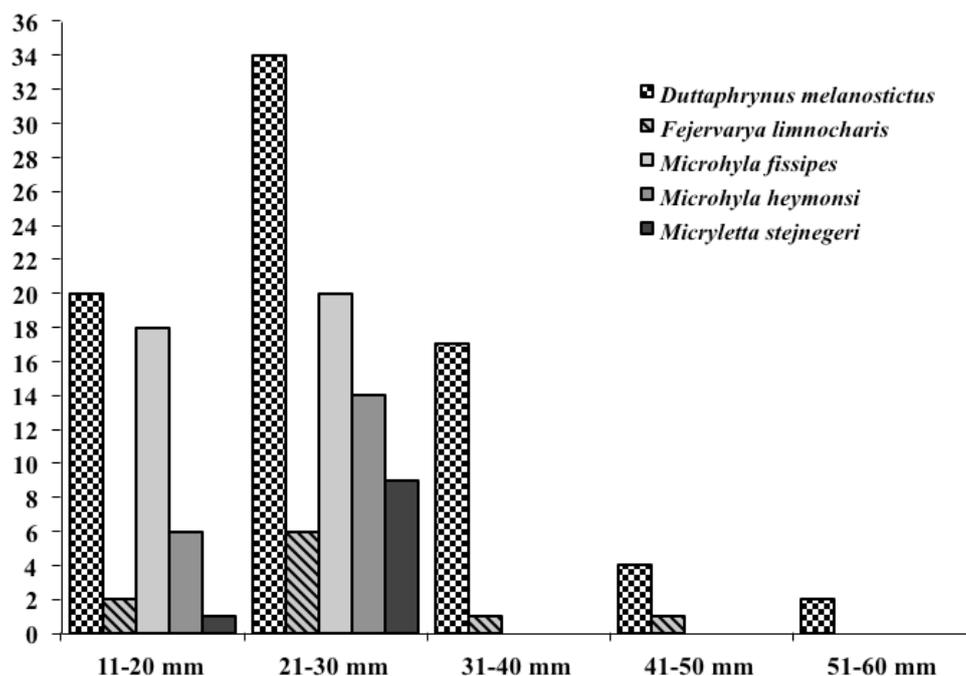


Figure 1. The numbers of *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymsi*, and *Micryletta stejnegeri* specimens used in this study, in 10 mm size classes.

MATERIALS AND METHODS

From January to November 2004, and again March to October 2006, pitfall traps were set in two betelnut palm (*Areca catechu* L.) plantations and a secondary forest in Santzepu, Sheishan District, Chiayi County, southwestern Taiwan (23°25'42"N, 120°29'06"E) to monthly sample arthropods, as part of studies to examine the impact of *Anolis sagrei*, an exotic invasive lizard species, on arthropod communities in this locality (Huang *et al.*, 2008a; Huang *et al.*, 2008b). Each pitfall trap consisted of four 500 ml cups that were planted in the soil so that the mouth of every cup was flush with the soil surface. The cups were arranged in a Y-shape, and three 1 m long plastic fences, with a height of ca. 300 mm, were placed between the four cups (Huang *et al.*, 2008b) to enhance the arthropod interception efficiency of the traps. During each survey, the pitfall traps were each filled with 200 ml of 70% alcohol, and were left open for seven consecutive days to collect terrestrial arthropods. For the study described herein, we utilized anurans that were accidentally collected by the pitfall traps.

Since the anurans were accidentally collected and were sometimes in a poorly preserved condition, and because the purpose of this study was to describe their diets, the genders of the specimens were not determined. Each anuran was treated as follows; the snout-vent length (SVL) was measured with a dial caliper to the nearest 1.0 mm, after which it was dissected by making a mid-ventral incision. We removed the stomach and slit it longitudinally, after which the stomach content was removed. The stomach contents were spread in a petri dish and examined under a dissection microscope, and all the prey items were identified, based on their morphology, either to the order or family (only for ants and spiders) level, and the numbers of all prey types were recorded. Due to the relatively small numbers of specimens of all species collected in each month, no inter-seasonal comparisons could be made, and all the data of each species was pooled.

Table 1. The numbers (*N*) of specimens of each species, as well as the ranges, means and standard deviations (\pm SD) of the snout-vent lengths (SVL in mm) of the anurans used in this study.

Species	<i>N</i>	Range	Mean \pm SD
<i>Duttaphrynus melanostictus</i> (Schneider, 1799)	77	12-52	26.4 \pm 8.8
<i>Fejervarya limnocharis</i> (Gravenhorst, 1829)	10	18-44	27.6 \pm 8.5
<i>Microhyla fissipes</i> Boulenger, 1884	39	11-25	20.4 \pm 2.8
<i>Microhyla heymonsi</i> Vogt, 1911	20	14-26	21.2 \pm 3.2
<i>Micryletta stejnegeri</i> (Boulenger, 1909)	10	13-26	21.8 \pm 3.6

Voucher anurans were deposited in the herpetology collection of the Natural History Museum of Los Angeles County (LACM), Los Angeles, California, U.S.A. (Appendix 1).

The software, Ecological Methodology Version 6.1 for Windows (Exeter Software, New York, USA), was used to determine the following. The diet diversity index of each species was measured with the Shannon-Wiener Function, and the Levin's Measure was used to estimate the niche breadth (Krebs, 1999). The Pianka's Measure and percentage overlap were used to examine the niche overlap among the species (Krebs, 1999).

RESULTS

The anurans used in this study, consisted of 156 specimens of five species, from the families, Bufonidae, Dicroglossidae and Microhylidae (tab. 1). The most frequently collected species was *Duttaphrynus melanostictus*, followed by *Microhyla fissipes*, *Microhyla heymonsi*, *Micryletta stejnegeri*, and *Fejervarya limnocharis*, in that order of frequency. The majority of specimens of all five species were relatively small (i.e. SVL \leq 30 mm) (fig. 1).

We recorded 3406 prey items, from 21 orders of 6 classes (tab. 2). Hymenopterans, of which the largest proportion were ants (Formicidae) (fig. 2), were the most numerous prey items in the diets of all five anuran species. Most of the anurans that preyed upon ants were the smaller (SVL \leq 30 mm) individuals, and their stomachs, on average, contained larger numbers of ants (tab. 3). Coleopterans and hemipterans were the only other orders present in the diets of all five anuran species. Spiders (Aranea) made up only a small proportion (ca. \leq 1%) of the consumed prey items, thus inter-species comparisons were not feasible. However, it is worth noting that spiders of

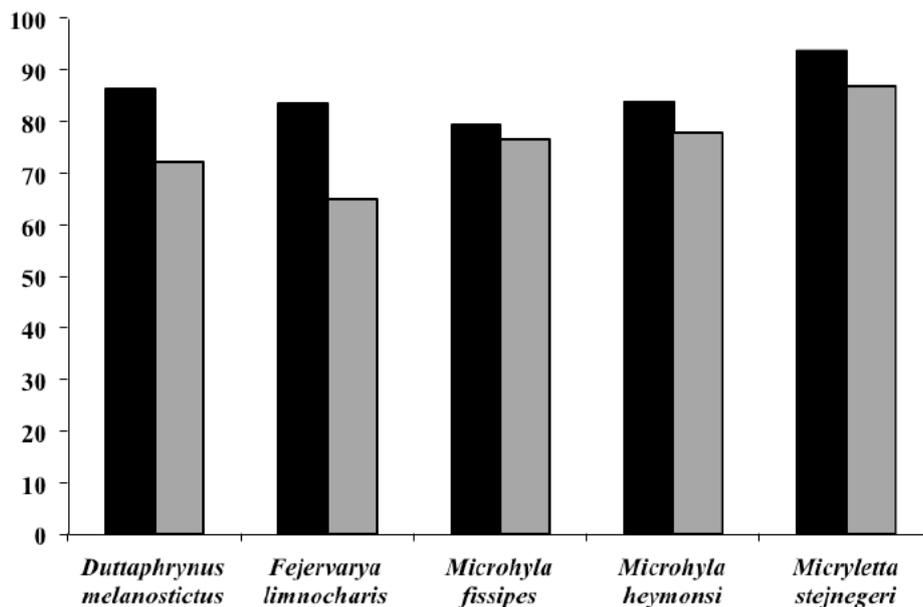


Figure 2. The ants (Formicidae), as a percentage of the total number of hymenopterans (black), and the ants as a percentage of the total number of prey items (gray), of the *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymonsi*, and *Micryletta stejnegeri* used in this study.

Table 2. The dietary composition (N; as a % of the total number of recorded prey items), and frequency of occurrence (F; the number of anurans, as a %, of each species, the prey type was recorded from) of the dietary items of the *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymonsi* and *Micryletta stejnegeri* used in this study. (Total = the total number of prey items).

Class	Order	<i>Duttaphrynus melanostictus</i>		<i>Fejervarya limnocharis</i>		<i>Microhyla fissipes</i>		<i>Microhyla heymonsi</i>		<i>Micryletta stejnegeri</i>	
		N	F	N	F	N	F	N	F	N	F
Arachnida	Acarina	0.06	1.3			0.1	5.13	0.28	5		
	Aranea	0.98	18.18	1.03	10	0.41	12.82			0.82	10
Chilopoda	Scolopendromorpha	1.84	6.49								
Clitellata	Haplotaxida	0.06	1.3								
Diplopoda	Spirobolida	0.4	6.49								
Gastropoda	Stylommatophora	2.07	14.29								
	Veronicelloidea	0.23	2.6								
Insecta	Blattaria	0.12	2.6			0.1	2.56				
	Coleoptera	4.26	55.84	2.05	20	1.23	25.64	1.38	25	3.28	20
	Collembola	1.5	9.09	5.13	30	0.41	5.13				
	Dermaptera	0.29	5.2	2.05	30	0.1	2.56				
	Diptera	0.17	3.9	2.05	20	0.1	2.56				
	Hemiptera	0.92	12.99	2.56	20	0.41	5.13	0.55	10	0.82	10
	Hymenoptera	83.71	87.01	77.95	80	96.83	94.87	92.82	95	92.62	100
	Isoptera	0.86	5.2	0.51	10			4.7	5		
	Lepidoptera	0.98	14.29	0.51	10						
	Neuroptera	0.12	2.6			0.1	2.56	0.28	5		
	Orthoptera	0.29	5.2	1.03	20					0.82	10
	Psocoptera	0.17	3.9	3.08	20	0.1	2.56			0.82	10
	Siphonaptera	0.06	1.3							0.82	10
	Trichoptera	0.06	1.3								
Unknown		0.86	9.09	2.05	30	0.1	2.56				
Total		1737		195		990		362		122	

the family Linyphiidae, were the most frequently recorded spiders in the stomach contents of the anurans described herein, followed by members of the family Theridiidae (fig. 3). The remaining identifiable spiders belonged to families that were infrequently recorded.

Based on the Shannon-Wiener Function, *Fejervarya limnocharis* had the highest prey diversity, followed by *Duttaphrynus melanostictus*, *Micryletta stejnegeri*, *Microhyla heymonsi*, and *Microhyla fissipes*, in that order (tab. 4). As for the niche breadth, *Fejervarya limnocharis* also had the broadest dietary niche breadth, followed by *Duttaphrynus melanostictus*, *Microhyla fissipes*, *Micryletta stejnegeri*, and *Microhyla heymonsi*, in that order (tab. 4). There were also substantial dietary overlaps among the anurans described herein, and the degree of overlap ranged from ca. 80%, between *Fejervarya limnocharis* and *Microhyla heymonsi*, to ca. 95%, between the three microhylid species (tab. 5).

DISCUSSION

Duttaphrynus melanostictus, formerly classified as *Bufo melanostictus* (Frost et al., 2006), and *Fejervarya limnocharis*, formerly known as *Rana limnocharis* (Dubois & Ohler, 2000), are both common species in lowland plains and agricultural areas in Taiwan (Yang, 1998). *Microhyla fissipes* (formerly classified as *Microhyla ornata*; Matsui et al., 2005) is a common species in lowlands and low montane regions all over Taiwan, and although *Microhyla heymonsi* is also common in most of the same areas, it is absent in the northern parts of the island (Yang, 1998). The distribution of *Micryletta stejnegeri* is restricted to lowland regions of central and southern parts of Taiwan, where it is infrequently encountered (Yang, 1998; Lue et al., 2002).

The inter-locality variations and similarities in the diets of *Duttaphrynus melanostictus* (Berry & Bullock,

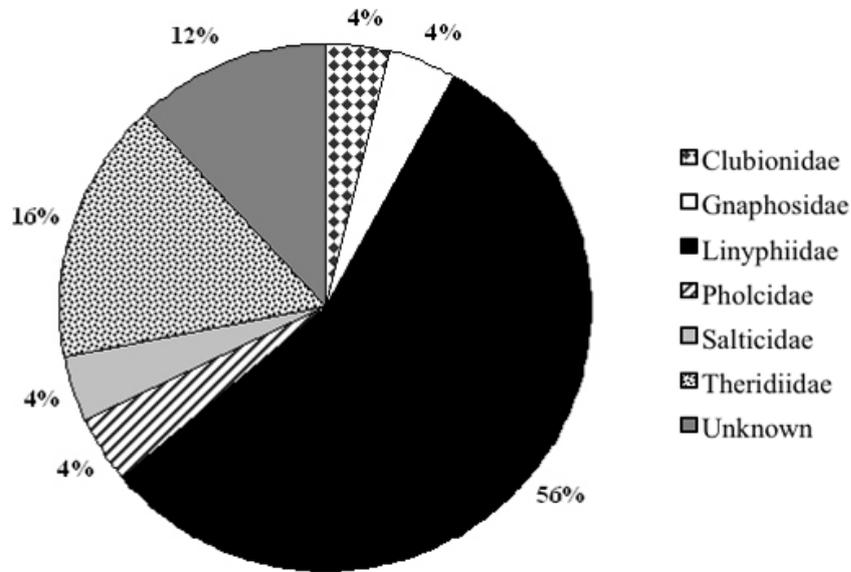


Figure 3. The proportions of the spider (Aranea) families recorded as prey of the *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymonsi*, and *Micryletta stejnegeri* used in this study.

1962; this study) and *Fejervarya limnocharis* (Berry, 1965; Wu *et al.*, 2007; Yu & Guo, 2012; this study) suggest, as stated by Berry (1965), that *Duttaphrynus melanostictus* and *Fejervarya limnocharis* are generalist predators that lack an apparent dietary preference, and that their diets are most likely dependent on what prey is available. As a bufonid, *Duttaphrynus melanostictus* is less dependent on water than *Fejervarya limnocharis*, and can thus occur in a larger variety of habitats. As a result, *Duttaphrynus melanostictus* can be expected to have access to a greater variety of prey types. In our study, *Duttaphrynus melanostictus* was the only species that preyed upon all the prey orders recorded. In spite of this, due to the dominance of ants in its diet, *Duttaphrynus melanostictus* has a lower prey diversity index than *Fejervarya limnocharis*. Toft (1980) stated that many species from the families Bufonidae, Microhylidae and Dendrobatidae are specialists, characterized by the preference of some arthropods (often Formicidae). In our literature review, we found only one other study describing the diet of *Duttaphrynus melanostictus* (Berry & Bullock, 1962), and in that study ants were also the most numerous prey items, followed by termites (Isoptera). Considering that most of the *Duttaphrynus melanostictus* specimens used in this study had a SVL smaller than 40 mm, and since ants were the most numerous collected arthropods in the study area (Huang *et al.*, 2008b), the results of our study is not out of the ordinary (see discussion below). Our results thus provides further support for the opinion of Berry and Bullock (1962) that *Duttaphrynus melanostictus* is an unselective feeder that will opportunistically prey on prey of suitable size. Of the three studies we found with references to the diet of *Fejervarya limnocharis*, ants were the most numerous prey items in two studies (Berry, 1965; Yu & Guo,

Table 3. The ranges, means and standard deviations in parenthesis (Total = the total number of prey items.), of the number of ants recorded in the stomachs of the *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymonsi*, and *Micryletta stejnegeri* specimens of various size classes.

	<i>Duttaphrynus melanostictus</i>	<i>Fejervarya limnocharis</i>	<i>Microhyla fissipes</i>	<i>Microhyla heymonsi</i>	<i>Micryletta stejnegeri</i>
11-20 mm	0-121 (18.4 ± 30.2)	3-5 (4.0±1.4)	0-63 (14.7 ± 18.4)	1-15 (6.0 ± 5.7)	0
21-30 mm	0-102 (14.3 ± 25.1)	0 - 112 (19.5 ± 45.3)	0-100 (24.8 ± 30.9)	0-81 (17.6 ± 28.9)	2-31 (11.8 ± 9.5)
31-40 mm	0-114 (22.4 ± 29.8)	0			
41-50 mm	0-10 (4.5 ± 5.3)	2			
51-60 mm	0				

Table 4. The Shannon-Wiener Function (H' = bits/individual; N_1 = units of numbers of species), Levin's Measure of niche breadth (B), and standardized Levin's Measure of niche breadth (B_A) of the diets of the anurans used in this study.

	Shannon-Wiener Function		Levin's Measure	
	H'	N_1	B	B_A
<i>Duttaphrynus melanostictus</i>	0.365	0.776	6.088	0.242
<i>Fejervarya limnocharis</i>	0.441	0.737	7.895	0.328
<i>Microhyla fissipes</i>	0.092	0.938	2.709	0.081
<i>Microhyla heymonsi</i>	0.144	0.905	2.139	0.054
<i>Micryletta stejnegeri</i>	0.165	0.892	2.651	0.079

2012), while orthopterans were the most numerous prey items in the third study (Wu *et al.*, 2007). This provides further support for the suggestion that *Fejervarya limnocharis* is a dietary generalist that preys on whatever prey is present in any given habitat (Berry, 1965). As for the microhylids, the species we examined are all fairly similar in size, and are substantially smaller than *Duttaphrynus melanostictus* and *Fejervarya limnocharis*. It is therefore not surprising that the prey of the microhylids were limited to mainly smaller types of prey. Only two dietary descriptions for *Microhyla heymonsi* were found (Berry, 1965; Erftemeijer & Boedi, 1991), and the results from our study included most of the prey categories also recorded in those studies. In studies into the diets of *Microhyla butleri*, *Microhyla heymonsi* (Berry, 1965), *Microhyla okinavensis* (reported as *Microhyla ornata*; Hirai & Matsui, 2000), and *Microhyla ornata* (Das, 1996), it was found that the diets of these anurans primarily consisted of ants. The results of those studies, as well as ours, support thus the conclusion of Hirai and Matsui (2000) that members of the genus *Microhyla* are ant-specialists.

An interesting finding of this study is the relatively high incidences of predation on ants and beetles by *Duttaphrynus melanostictus*, *Microhyla fissipes*, *Microhyla heymonsi* and *Micryletta stejnegeri*. Ants are generally considered to be of poor nutritional value (Redford & Dorea, 1984; Withers & Dickman, 1995; Meyers & Herrel, 2005). Many ants, beetles and some other arthropods, such as millipedes, also produce noxious chemicals that make them unpalatable to many potential predators (Skaife, 1979). However, *Duttaphrynus melanostictus* readily feeds on arthropods, such as ants, centipedes and millipedes that contain noxious chemicals. Some anurans actually incorporate the noxious chemicals produced by such arthropods into their own defensive mechanisms, and thus selectively prey on such invertebrates (Daly *et al.*, 1994a, 1994b, 2007). Skin secretions of *Duttaphrynus melanostictus* contain neurotoxins and cardiotoxins (Das *et al.*, 1998). Whether *Duttaphrynus melanostictus* incorporates the noxious chemicals produced by some prey as toxic skin secretion, is not clear, and deserves further empirical study. The potential that predation on ants by *Microhyla fissipes*, *Microhyla heymonsi* and *Micryletta stejnegeri* may also play a role in their defensive mechanisms should also be explored.

In a study into the diets of an anuran community in India Das (1996) found that in prey selection, prey sizes were more important than prey types. The author suggested that such a trend may be generally true for insectivorous predators that ingest their prey whole. Although we did not measure the sizes of the prey items we obtained from the stomachs of the anurans in our study, the dietary results still seem to suggest a similar trend. All the anuran specimens in whose stomachs we recorded spiders belonging to the family Linyphiidae were relatively small (SVL \leq 35 mm). The same can be said about the anurans that preyed upon mite (Acarina). The dominant ants in the habitat are *Pheidole fervens*, *Paratrechina kraepelini*, and *Pachycondyla luteipes* (Huang *et al.*, 2008a), and all these species are only a few millimeters in length. Ants were the most numerous prey items of the smaller examined frog specimens (SVL \leq 40 mm), while they were less frequently recorded in larger specimens (SVL $>$ 40 mm). Since ants and spiders of the family Linyphiidae were the most numerous collected insects and spiders in the study area (Huang *et al.*, 2008a, 2008b), their high frequency in the stomachs of small anurans, but low frequency in larger anurans, strongly suggest that the latter have a preference for larger prey.

It is also worth noting that most of the common insects and spiders, recorded in the studies done in the area where the frogs were collected (Huang *et al.*, 2008a, 2008b), were also present in the diets of the anurans discussed herein. However, the numbers collected do not reflect those removed from the stomachs of the frogs. Even though hymenopterans were the most numerous insects collected and also the most frequently recorded prey items, the second most frequently collected insects were lepidopterans (Huang *et al.*, 2008a, 2008b), which appeared only infrequently in the diets of the larger anuran species. As for the spiders, a similar trend was observed. Members of the family Linyphiidae, which were the most numerous collected specimens (Huang *et al.*, 2008b), were also the most frequently recorded spiders in the diets of the frogs. However, members of the second most numerous collected spider specimens belonged to the family Salticidae (Huang *et al.*, 2008b), which was recorded infrequently

Table 5. The Pianka Measure (un-shaded area) and percentage dietary niche overlap (gray shaded area) of the *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Microhyla fissipes*, *Microhyla heymonsi* and *Micryletta stejnegeri* used in this study.

	<i>Duttaphrynus melanostictus</i>	<i>Fejervarya limnocharis</i>	<i>Microhyla fissipes</i>	<i>Microhyla heymonsi</i>	<i>Micryletta stejnegeri</i>
<i>Duttaphrynus melanostictus</i>		0.996	0.998	0.998	0.999
<i>Fejervarya limnocharis</i>	86.2		0.996	0.995	0.996
<i>Microhyla fissipes</i>	86.8	80.8		0.999	1.000
<i>Microhyla heymonsi</i>	86.7	80.4	94.7		0.998
<i>Micryletta stejnegeri</i>	89.1	83.3	94.8	94.6	

in the diets of the anurans. The most plausible reason for this result is that the anuran species used in this study are strictly terrestrial, and their diets would naturally also be restricted to prey they encounter on the ground (i.e. ants, terrestrial spiders, etc.). Another aspect that should be taken into consideration is activity patterns. While the anuran species used in this study are primarily nocturnal, some of the collected arthropods, such as wolf spiders (Lycosidae), are often active during the day and as a result are absent in the diets of the frogs. The results of this study thus indicate that these anurans are primarily predators of nocturnal terrestrial arthropods.

Among the anuran species described herein, there were some variations in the prey they consumed, which is most likely a result of their respective builds and/or habitat preferences. Still, at the order level, there were substantial dietary niche overlaps among the anurans used in this study. Since this study utilized specimens that were accidentally collected on an ad hoc basis, and because the majority of the prey items were only identified to the order level, the data is insufficient for detailed dietary competition descriptions. Future dietary niche partitioning and competition studies should attempt to identify the major prey categories to the species, or at least genus levels, because there may be variations in the species, even of the same order, in the diets of anurans.

Our additional information pertaining to the diets of the five anuran species discussed herein not only contributes to the understanding of the natural history of these frogs, but also indicates their ecological role as predators. The studies into the impact of *Anolis sagrei* on arthropods in the area where our specimens were collected, found that these lizards reduced the numbers of certain arthropods (Huang *et al.*, 2008b) and altered the ant community structure (Huang *et al.*, 2008a). Considering the large numbers of prey the anurans in our study consumed, it is likely that they also impact terrestrial arthropod communities in this habitat. In particular, the impact on ant communities by the small species, such as the microhylids, and the sub-adults (i.e. smaller sized individuals) of the bigger species, deserve further empirical study.

ACKNOWLEDGEMENTS

The research presented herein adhered to the legal requirements of Taiwan, R.O.C. Since the species used are not listed as protected species in Taiwan, and because the collection was not done in a national park or other conservation area, no collection permit or other documentation was required. The authors would like to express their gratitude to Dr. David C. Blackburn and the other anonymous reviewer, whose comments helped improve this report.

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APPENDIX 1

Some of the anuran specimens, and all the stomachs and stomach contents were accidentally lost. The remaining anuran specimens were deposited in LACM as *Duttaphrynus melanostictus* LACM 182805-18210, 182815, 182825, 182843; *Fejervarya limnocharis* LACM 182816-182818, 182824; *Microhyla fissipes* LACM 182811, 182812, 182826, 182827, 182840, 182844; *Microhyla heymonsi* LACM 182819-182821, 182823, 182839; *Micryletta stejnegeri* LACM 182838.