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Diet Composition of Six Anuran Species (Amphibia: Anura) in Terminalia Forest, Mindanao Island, Philippines

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Abstract

The diet of six anuran species (*Kaloula conjuncta*, *Philautus acutirostris*, *Polypedates leucomystax*, *Occidozyga laevis*, *Fejervarya cancrivora* and *Rana granducola*) was determined using the gut content analysis. Nineteen food items were identified with Hymenoptera: Formicidae as the most frequently occurring food item appearing in the guts of 5 out of 6 species. Coleoptera, Diptera, and Orthoptera followed appearing in 4 out of 6 species. The constantly frequent occurring food item for each species was: Hymenoptera for *K. conjuncta* and *P. leucomystax*; Hemiptera for *O. laevis*; and Orthoptera for *R. grandocula*. Degree of food preference (DFP) differed for each food item. Preferential food items (2<DFP<3) were Hymenoptera for *K. conjuncta* and *P. leucomystax* and Orthoptera for *R. granducola*. Presence of accidental occurrence and occasionally preferred food items was prominent in *P. acutirostris*. The occurrence of tadpole in the gut of *O. laevis* suggesting batracophagy is reported here. The recovery of food items that serve as bioindicators for water quality suggest that the water quality in the area was good. Thus, this study strongly supports the protection of the area for conservation of both anurans and their prey.

Keywords: gut contents, invertebrates, Sago stands, visual encounter technique

Abbreviation:
DFP – Degree of Food Preference: an index that measures the consumption of a particular food group
FOO – Frequency of Occurrence: a measure of dietary composition where the number of times each food item appeared in stomachs was noted

Introduction

The natural diet of adult amphibians has been reported to include a wide variety of small insects and other invertebrates such as mollusks, annelids, centipedes, millipedes, arachnids and crustaceans (Durell Wildlife, 2005). Some, especially the larger ones have also been documented to prey on small mammals, birds and even other anurans. The group may be described as specialists or generalists, feeding by ambush or foraging (Stocker, 2000).

For tropical anurans, two main diet patterns have been identified by Toft (1980a, 1981 as cited in Santos et al., 2004): the "ant specialists" and the "non-ant specialists". The "ant specialists" are those observed to eat more chitinous, slow-moving arthropods while the "non-ant specialists" are those which consume larger, less chitinous and more mobile arthropods such as Orthopterans and large spiders.

In the Philippines, the limited literatures on herpetology focussed especially on species composition and dietary information of adult anurans is really lacking. For instance, the first major systematic revision of Philippine Amphibia done by Inger in 1954 had focused primarily on the distributional pattern exhibited by amphibians. Also, although the earlier works of Brown and Alcala (1955, 1961, 1964, 1986) had further supplemented data on Philipine herpetology, these reports were mostly centered on amphibian species composition and distribution. In few Philippine species that have been studied for their food preference, they were reported to be carnivorous and insectivorous in food habit. These anurans that were analyzed include the species of *Kaloula picta*, *K. conjuncta*, *Occidozyga laevis*, *Limnonectes magnus*, *Rana microdisca*, *R. erythraea*, *Fejervarya cancrivora* and *Platymantis sp.* Except for *R. erythraea* and *F. cancrivora* which may assume a cannibalistic habit, the rest of the anurans mentioned were reported to exhibit an "ant-specialist" diet pattern (Alcala, 1976; Alcala and Brown, 1998; Toft, 1980a, 1981 as cited in Santos et al., 2004).

The importance of dietary information in understanding the impact of habitat modifications on fluctuating populations is well known. This is true for species that inhabit endangered areas (Anderson et al., 1999 and Conservation International do Brasil, 2000 as cited in Santos et al., 2004). Thus, dietary information would be useful in the Philippines which supports not only a high level of amphibian endemism but is also one of the most biodiversity endangered areas of the world (Conservation International, 2006).

This study was conducted to examine the diet composition of six anuran species sampled from a Terminalia forest adjacent to a *Sago* forest. Specifically, this study documented the: a) food items found in the gut of anurans through stomach content analysis and b) identified the degree of preference and frequency of occurrence of each food item.

Materials and Methods

Collection site

The frog samples used in this study were collected from a Terminalia forest in Sitio Kaliloan, Barangay Poblacion, Bunawan, Agusan del Sur, Mindanao, Philippines located at lat 8°10'02"N, long 125°59'57"E (Figure 1). Collection site is on the opposite side of Mihaba river. The area is dominated by stands of Terminalia but

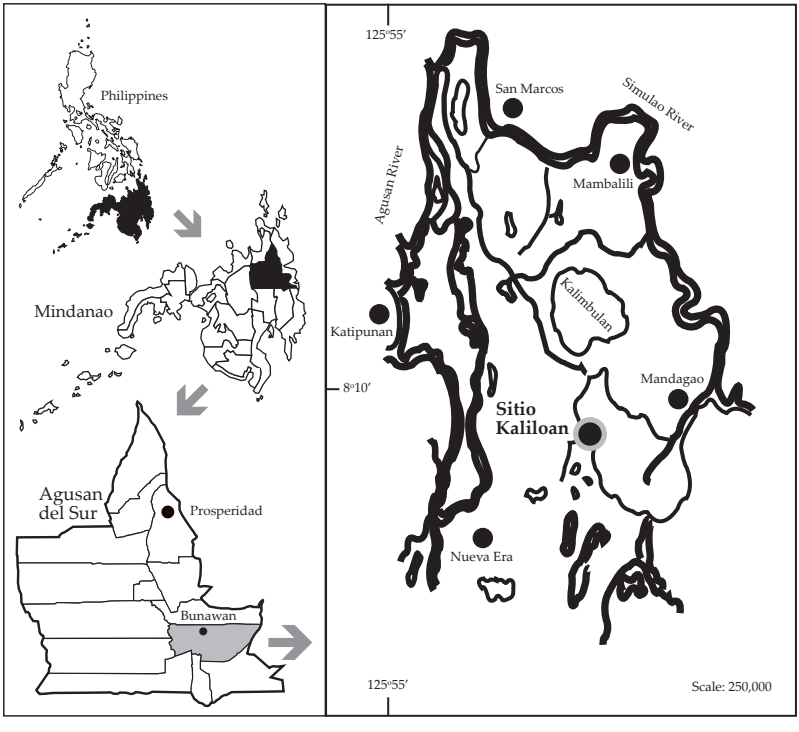

Figure 1. Map of the Philippines, Mindanao, and Agusan del Sur (inset) showing the location of Sitio Kaliloan, Agusan del Sur (Source: Assessors Office, Municipality of Bunawan)

other trees such as *Narra* are also present. Ferns, grasses and various vines can also be found in the site. It is also surrounded with clearings mainly for agricultural activities. *Sago* stands are also seen adjacent to the collection site. Average annual rainfall in the area is 4,286 mm. It has a mean annual temperature of 25.6 °C and a mean annual relative humidity of 86%. Samples were collected in April to May 2004 and in October 2005 (Table 1 and Figure 2a-f) through the visual encounter technique where a 2-kilometer transect line was established along known trails. Approximately 5 m on each side of the transect was searched between 0900–1400 h and 1800–2100 h. The collected samples are currently deposited at the Molecular Biology Laboratory of the College of Science and Mathematics of the University of the Philippines Mindanao (UP Mindanao).

Diet composition

All samples were dissected and examined in the Biology Laboratory of UP Mindanao. Prior to dissection the size, sex and maturity of the samples were noted. The stomach was removed and opened and the contents were scraped out and examined under the microscope. The degree of prey digestion was estimated as follows: A) fresh – whole organism relatively still intact; B) intermediate – body and most flesh intact; other body parts may be digested; C) immeasurable body parts only – parts cannot be reconstructed to obtain standard measurements, but higher taxon or species group still identifiable; and D) Fully digested – unidentifiable material, slurry; amorphous substance. Identifiable prey items were enumerated, counted and categorized into food groups

Quantification of stomach contents

Dietary composition was quantified using the frequency of occurrence (FOO) method wherein each food item was noted as present or absent in each stomach. The frequency at which different food categories appeared in stomachs was calculated for each species as shown:

$$\text{Frequency of Occurrence} = \frac{\text{Number of stomachs an item appeared} \times 100}{\text{Total number of stomachs with content}}$$

Food items were then regarded as Constant when registered in > 50% of the stomachs of a particular species, Secondary when present in 25-50% of stomachs or Accidental when observed in <25% of the stomachs (Dajoz, 1983 as cited in Santos et al., 2004).

Table 1. Comparison of snout-vent length (SVL) of anurans collected in Terminalia Forest, Sitio Kaliloan, Barangay Poblacion, Agusan del Sur between 2004-2005 and used for stomach content analysis with that reported in literature

Species	SVL (cm) X ± SD	Number of adults analyzed	SVL (cm) (Alcala and Brown, 1998)
Microhylidae			
Kaloula conjuncta	4.05 ± 0.8	4	2.9-4.7
Ranidae			
Occidozyga laevis	4.30 ± 0.90	9	2.6-6.2
Rana grandocula	6.12 ± 1.43	5	3.1-7.5
Rana cancrivora	5.84 ± 1.14	18	4.6-8.8
Rhacophoridae			
Philautus acutirostris	2.53 ± 0.4	33	1.6-28
Polypedates leucomastyx	5.26 ± 0.65	7	5.0-7.5
Total		76	

X = mean, SD = standard deviation

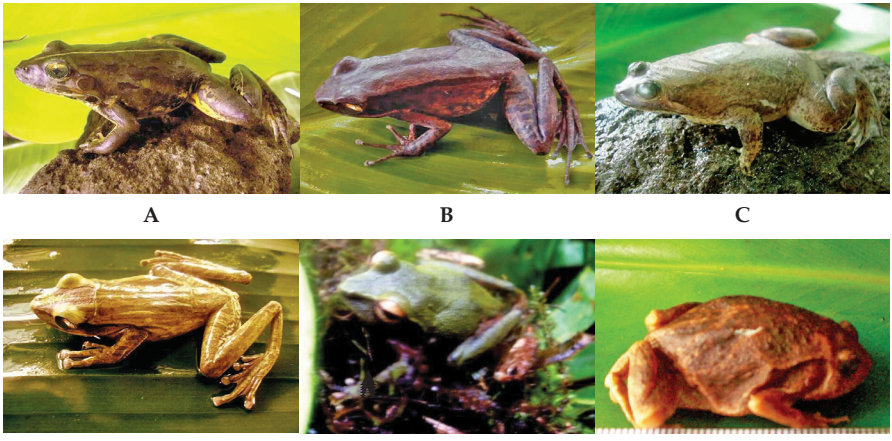

Figure 2. Frog species collected in Sitio Kaliloan, Agusan del Sur: a. *Fejervya cancrivora*; b. *Rana grandocula*; c. *Occidozyga laevis*; d. *Polypedates leucomystax*; e. *Philautus acutirostris*; and f. *Kaloula conjucta*

Degree of food preference

Degree of food preference (DFP) index developed by Braga (1999 as cited in Santos et al., 2004) was used to infer the consumption of a particular food item. Food was ranked by categories from 1 to 4: the maximum value (4) was assigned when only one food item was found in a stomach. When stomachs contained more than one group,

the value "3" was given to the most abundant, while "2" was given to the second most common, and "1" was attributed to the less abundant group. Abundance was estimated by the number of prey items of each category in each stomach. DFP was calculated as follows: DFP = S*(i)*/ N: where S*(i)* is the sum of values given to a food item *i* in the guts and N is the total number of guts of each species analyzed. Food items were then categorized as highly preferential (3<DFP<4), preferential (2<DFP<3), secondary (1<DFP<2) or occasional (0<DFP<1).

Results and Discussion

Diet composition

Of the 76 anuran guts examined only 55 had gut contents. Some of the adults examined could have defecated and have not eaten prior to capture thus reducing the number of samples with filled stomachs. Finding samples with empty stomach was also hypothesized to be related to sex (males being too busy to eat as they are in constant search for a mate), seasonality (mating season or season of low food abundance) or due to highly patchy distribution of food within the area (Biavati et al., 2004). Nineteen food items were identified and were broadly classified into four groups: invertebrate, vertebrate, plant matter and unidentifiable food group (Table 2). These include 14 invertebrates, two vertebrates and two plant matter. The unidentified category consist mostly of unidentifiable fragments which are mostly invertebrates. The degree of digestion of gut contents ranged from fresh to amorphous substances.

Among the food items, Hymenoptera (five out of six species), Coleoptera, Diptera and Orthoptera (four out of six species) were frequently encountered in the stomachs of the samples. The Hymenopterans in this study were represented by both winged and wingless black and red ants and these were recorded in all species except *R. grandocula*. The wingless ants appear to be consumed more than winged ones. The winged ants were partly digested when excised from anuran guts with their wings detached from the body. In addition, digested ants were still identifiable through their detached head and abdomen as their body parts are sclerotized and need longer time to be digested. Similar findings on ants being the dominant food item were also reported among the female samples of the microhylid frog *Chiasmocleis capixaba* of Brazil (Vansluys et al., 2006).

Meanwhile, the coleopterans excised were mostly beetles including larva; the dipterans identified were mosquitoes and flies while grasshoppers were the main orthopterans excised from the gut of

Table 2. Food items found in the stomach of anurans collected at the Terminalia Forest, Sitio Kaliloan, Barangay Poblacion, Bunawan, Agusan del Sur

Food items	Kc	Ol	Rg	Fc	Pa	Pl
Invertebrates						
Arachnida		B	B		B, C	
Embiidina	B				A, B	
Coleoptera	C, B	C		A	A, B, C	
Beetle larva		A			B	
Dermaptera				A, C		
Diptera		B		B	B	C
Ephemeroptera					C	
Hemiptera	A	A, B			A, B	
Hymenoptera: Formicidae	A, C	C		A	B, C	A, C
Lepidoptera (larvae)		B	A		A, C	
Odonata: zygoptera		A				
Orthoptera		C	B, C	B	B, C	
Mollusca		A			A	
Protozoa				A		
Vertebrates						
Amphibian tadpole		A				
Fish matter					C	
Unidentified		C		D	C, D	D
Plant matter						
Seed				A		
Leaf		C		B	C	

Species: Kc – *Kaloula conjuncta*; Ol – *Occidozyga laevis*; Rg – *Rana grandocula*; Fc – *Fejervarya cancrivora*; Pa – *Philautus acutirostris*; Pl – *Polypedates leucomystax*;
Degree of prey digestion: **A** – fresh, whole organism; **B** – intermediate, body and most flesh intact; **C** – Immeasurable body parts, higher taxon still identifiable; **D** – fully digested, slurry, amorphous substance

the anurans. Also included in the diet are arachnids, lepidopterans and hemipterans which were found in three out of six species. The rest of the food items excised were observed in the stomachs of two species (food items Embidiina and Mollusca) or in one species only (food items Dermaptera, Odonata: Zygoptera, amphibian tadpole and fish matter).

The tendency for arthropods and other social insects to be overestimated in the analysis of gut contents had been observed by Measey et al. (2004) in the soil-dwelling caecilian *Gegeneophis ramaswamii*. Measey et al. (2004) reported that arthropods, being recovered from the more posterior parts of the gut because of their relatively indigestible chitinized exoskeleton, may be prone to overestimation. Furthermore, the soil-dwelling social insects (termites and ants) ranked higher in frequency compared to the other gut contents excised. Thus, it is possible that the greater representation of the arthropod group especially ants in this study is also due to its relative indigestibility.

Frequency of occurrence

Diet composition varied among the six anuran species studied (Table 3). Food items were relatively similar yet vary in the frequency of occurrence (FOO). Invertebrate materials were the most frequently occurring food item in the stomach of the anurans. This may imply that invertebrates appear abundant in the area and are the immediate food item frogs could get easy access. In contrast, vertebrate items appear scarce and difficult to be considered as a prime diet due to its bigger size relative to the frog thus, are only recorded in lower frequency.

Among the invertebrates, Hymenoptera: Formicidae was the most frequently occurring invertebrate material found in the guts of five out of six anuran species. It was a constant food item in the stomach of *K. conjuncta* (100%) and *P. leucomystax* (67%) but was only accidental for *O. laevis* (17%), *F. cancrivora* (18%) and *P. acutirostris* (11%). The

Table 3. Frequency of occurrence (%) of food items found in the stomach of anurans collected at the Terminalia Forest, Kaliloan, Barangay Poblacion, Bunawan, Agusan del Sur

Category of food item	Frequency of Occurrence (%)					
	Kc	Pa	Pl	Ol	Rc	Rg
Invertebrates						
Arachnida		17[A]	33[S]		11[A]	
Embiidina	25[S]				11[A]	
Coleoptera	50[S]	17[A]		27[S]	18[A]	
Beetle larva		33[S]				
Dermaptera				45[S]		
Diptera		17[A]		9[A]	4[A]	33[S]
Ephemeroptera					4[A]	
Hemiptera	25[S]	67[C]			21[A]	
Hymenoptera:Formicidae	100[C]	17[A]		18[A]	11[A]	67[C]
Lepidoptera larvae		17[A]	33[S]		14[A]	
Mollusca		17[A]			4[A]	
Odonata: zygoptera		17[A]				
Orthoptera		17[A]	67[C]	36[S]	18[A]	
Protozoa				9[A]		
Vertebrates						
Amphibian tadpole		17[A]				
Fish					4[A]	
Unidentified		17[A]		9[A]	7[A]	67[C]
Plant						
Seed				9[A]		
Leaf		17[A]		18[A]	11[A]	
Number of Stomachs examined	4	9	5	18	33	7
Number of Stomachs with content	4	6	3	11	28	3

Species: Kc – *Kaloula conjuncta*; Ol – *Occidozyga laevis*; Rg – *Rana grandocula*; Fc – *Fejervarya cancrivora*; Pa – *Philautus acutirostris*; Pl – *Polypedates leucomystax*;
Frequency of occurrence: **C** (constant) - >50%; **S** (secondary) – 25 - 50%; **A** (accidental) - <25%.

high frequency of occurrence recorded for ants (Hymenopterans) in this study could be explained by their abundance in the sampling site. This group has also been reported previously occupying a wide range of habitats including *sago* areas and its vicinities (Cuenca 2004, unpublished). Similarly, Alcala (1976) also reported ants as predominating in the diets of *K. picta* and *K. conjuncta* and were also present in the guts of *O. laevis*, *R. microdisca*, *L. magna* and *Platymantis sp.* The higher occurrence of ants as diet of five out of six species may imply that majority of the species in study are ant specialists. However the presence of other taxon as component of the diet further attests to the opportunistic nature of anurans as predators (Santos et al., 2004).

The second frequently occurring food items were those belonging to Orders Coleoptera, Diptera and Orthoptera, which were all found in the guts of four out of six anuran species. Diptera was recovered in the gut of *P. leucomystax* (67%), Coleoptera from the gut of *K. conjuncta* (25%) and *F. cancrivora* (27%) and orthopteran samples were found in the guts of both *F. cancrivora* (36%) and *R. grandocula* (67%). Dipterans, coleopterans and orthopterans were also found abundant in the area hovering around *sago* inflorescence (Cuenca, 2004). Their abundance may also explain why they are commonly found in the guts of most anurans.

The least occurring food items in the sample were Protozoa, Odonata: Zygoptera, Embiidina, Ephemeroptera, fish, amphibian tadpole and plant seed. They were classified as accidental food items since their FOO value was <25%.

The occurrence of plant materials in the anuran gut can signify that plants have only been accidentally included in their feeding, since the plant material was always accompanied by invertebrates that inhabits plants. The Orthoptera (grasshoppers), for example, is usually found atop grasses and plant leaves, thus the guts of *R. cancrivora*, which constantly feeds on Orthopteran, have also a relatively higher incidence of plant material.

Food items with a high frequency of occurrence presented here are mostly those with sclerotized body parts including hymenopterans and coleopterans which are more difficult to digest than other possible soft-bodied food item eaten by frogs. Thus, hymenopterans and coleopterans are more identifiable than other food items. Some food items were regarded as unidentified thus it is possible that some insect groups especially those which are easy to digest may have been underestimated. In this regard, a definite conclusion on preferred food item based on frequency of occurrence may be difficult to draw.

Degree of food preference

For the degree of food preference (DFP), items were mostly considered occasional for most of the anuran species (Table 4). For anuran species with fewer samples, the food items were categorized as secondary or preferential food items. *O. laevis* and *P. acutirostris* had the most diverse diet with 13 food items, followed by R. cancrivora with 9 food items. *P. leucomystax*, *R. grandocula* and *K. conjuncta* had the most limited food item which ranged from 3-4 food items.

Preferential category was the highest level of DFP given to the food items of anurans. Family Formicidae of Order Hymenoptera and Orthoptera fell under this category. Formicidae was preferential for both *K. conjuncta* (2.50) and *P. leucomystax* (2.33).

Secondary preferential food items were from orders Arachnida, Coleoptera, beetle larva, Dermaptera, Hemiptera and Orthoptera. Although the abundance of these arthropod groups in Agusan del

Table 4. Degree of Food Preference (DFP) by anurans collected at the Terminalia Forest,Kaliloan, Barangay Poblacion, Bunawan, Agusan del Sur

Category	Kc	Pa	Pl	Ol	Rc	Rg
Number of stomachs examined	4	33	7	9	18	5
Number of stomachs with content	4	28	3	6	11	3
Food items						
Invertebrates						
Arachnida		0.39 o	0.50 o		1.00 s	
Embiidina	0.75 o	0.43 o				
Coleoptera	1.25 s	0.71 o		0.50 o	0.91 o	
Beetle larva				1.00 s		
Dermaptera					1.45 s	
Diptera		0.11 o	0.67 o	0.33 o	0.18 o	
Ephemeroptera		0.11 o				
Hemiptera	0.75 o	0.71 o		1.83 s		
Hymenoptera:Formicidae	2.50 p	0.39 o	2.33 p	0.33 o	0.55 o	
Lepidoptera larvae		0.46 o		0.50 o		1.00 s
Mollusca		0.11 o		0.50 o		
Odonata: zygoptera				0.33 o		
Orthoptera		0.64 o		0.50 o	1.27 s	2.67 p
Protozoa					0.27 o	
Vertebrates						
Amphibian tadpole				0.67 o		
Fish		0.11 o				
Unidentified		0.25 o	2.00 p	0.33 o	0.27 o	
Plant						
Seed					0.27 o	
Foliage		0.32 o		0.50 o	0.55 o	

Species: Kc – *Kaloula conjuncta*; Ol – *Occidozyga laevis*; Rg – *Rana grandocula*; Fc – *Fejervarya cancrivora*; Pa – *Philautus acutirostris*; Pl – *Polypedates leucomystax*;
Degree of Food Preference: **hp**-highly preferential (3<DFP<4); **p**-preferential (2<DFP<3); **s**-secondary(1<DFP<2); **o**-occasional(0<DFP<1)

Sur is not well-documented, their ubiquitous distribution is well established. Also, the palatability of these organisms as food for anurans had been observed in other species inhabiting the Central Amazon (Lima, 1998 as cited in Santos et al., 2004) as well as in other Philippine anurans (Alcala, 1976), such that their occurrence as secondary preferential food item in the six species studied here may be expected.

Occasionally preferred food items included all invertebrate food items except beetle larva and Dermaptera. The vertebrates (fish and tadpoles) were also categorized as occasional food items.

Alcala and Brown (1998) reported that most Phillipne anurans were invertebrate feeders and preying on fish has not yet been documented. Anurans are gape-limited predators that usually ingest their prey whole. Being gape-limited, they will tend to feed only on prey as large as they can swallow whenever they have the chance (Toft, 1985). Considering the size of the frog *P. acutirostris* (SVL: 2.53 cm) where the fish vertebra was recovered and that only a segment of it was excised, the occurrence of such food item may really only be considered accidental.

The presence of amphibian tadpole in the stomach of *O. laevis* is an interesting result although it was already reported earlier as possibly part of *O. laevis* diet (Villadolid and del Rosario, 1929). Although it appeared in only one *O. laevis* gut, its occurrence cannot be underestimated since there were four intact tadpoles excised. It was categorized as accidentally occurring and occasionally preferred food item, yet there is a need to further study the habit of this species to clarify the batracophagic behavior that it had exhibited.

The presence of plants in the anuran gut is also worthy taking note of. Santos et al. (2004) pointed out that information on plant consumption contributes to the understanding of behavioral patterns. They further stressed that the presence of anthers, stamens, pollens and leaves in anuran stomachs indicates that vegetation is used not only as a reproductive site but as a foraging territory as well. Previous reports on possible amphibian herbivory had already been cited by Santos et al. (2004) suggesting that plants may serve as a significant component of adult anuran diet. In this study, plant matter was recovered in three out of six species and although classified as accidentally occurring food item, it had appeared in comparative amounts with other food items.

In general, results show that invertebrate material was the preferential food item of the anurans studied. A remarkable variation in prey morphology, behavior and developmental stage has been

observed in the food items consumed suggesting variations in anuran habitat occupation. For instance, *P. acutirostris* inhabits arboreal and occasionally terrestrial microhabitats (Alcala and Brown, 1998) yet aquatic food items (mollusks and fish matter) were excised from its gut implying that the species may also dwell in aquatic microhabitats. Such variations in prey consumption were also observed by Santos et al. (2004) in other species of anurans indicating that data on diet composition can support ecological and behavioral field studies.

Conclusions

This study broadly identified and classified the food items found in the guts of six species of anurans. Although species samples were limited, an initial assessment of species' actual diet can be done. As a higher occurrence of ants had been observed from the guts of 5 out of 6 species, results may imply that majority of the species examined are "ant specialists." This conforms to previous reports that tropical anuran diet falls into two main diet patterns: the "ant specialists" and the "non-ant specialists." However, since the food items with high frequency of occurrence presented are mostly those with sclerotized body parts which are more difficult to digest, it is likely that other food items may have been underestimated. Thus, the data presented can not strongly designate specific food items preferred by each species.

Moreover, the occurrence of tadpoles in the gut of *O. laevis* is an interesting result reinforcing the possibility of a batracophagic behavior exhibited by this species. The occurrence also of aquatic food items in *P. acutirostris* suggests that it may also spend time near bodies of water. Lastly, the occurrence of bioindicators of good water quality (freshwater molluscs, riffle beetle larva, and Ephemeroptera) suggests that the area still has a good water quality and must be conserved.

Implication to Conservation

The diversity in the food items consumed by anurans examined in this study implies that anurans exploit differing habitat occupations. This indicates that the Terminalia forest and the adjacent *sago* stands in Sitio Kaliloan with their structural complexity, afforded amphibians a wide range of suitable microhabitat to exploit. This further indicates that these areas in Agusan Marsh, given their natural setting, are providing amphibians sufficient cover and diverse foraging sites

thus making the area favorable for their existence. Unfortunately, this site, like most other forested sites in the Philippines, is still subjected to habitat destruction thus endangering the survival of species inhabiting it. It is then on this premise that this paper raises the crucial role of the Terminalia forests to the survival of amphibians. The services that the area has provided not only to amphibians but to other vertebrates as well is vital to their existence. Thus, this paper strongly supports the conservation of this marginal area in Agusan Marsh.

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