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Preliminary Survey and Diet Analysis of Anurans in The Riparian Zone of Calayagon Watershed, Agusan Del Norte, Philippines

PRELIMINARY SURVEY AND DIET ANALYSIS OF ANURANS IN THE RIPARIAN ZONE OF CALAYAGON WATERSHED, AGUSAN DEL NORTE, PHILIPPINES

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ABSTRACT

Watersheds are critical habitats for a diverse array of organisms. Among all the fauna, anurans are excellent biological indicators of environmental health. The community structure is often associated with a relationship between species diversity and diet. An anuran survey was conducted along riparian zones of three selected barangays of Calayagon Watershed (Guinabsan, Rizal, and Malpoc), Philippines. Extensive opportunistic methods for a total of 480 man-hours were spent traversing the area. A total of 195 individuals, consisting of seven species from four families, were recorded. Seventy-two percent of the individual species were regarded as Least concern, and 14 % were Near-threatened. Of the species recorded, 57 % are Philippine endemic, and 29 % are Invasive alien species. Fejervarya vittigera (44.66 %) gained the highest number of individuals across sampling stations. Overall diversity index is high H'= 1.43. The most abundant species in each sampling site were subjected to diet analysis (*R. marina, F. vittigera,* and *F. moodiei*). The most dominant prey item were plant matters followed by insect orders. In terms of the number of prey items, the order Hymenoptera was the most abundant. The study was the first to record the diet of the Philippine endemic *F. vittigera,* and *F. moodiei*. Noted anthropogenic threats include agricultural expansion and urbanization. Strict implementation of ordinances and policies towards the conservation and protection of a healthy bio-system for anurans and all organisms in the area is highly recommended.

Keywords: Anuran diversity, calayagon watershed, diet analysis, Philippines.

INTRODUCTION

The Philippines is recognized as one of the most important centers of herpetofaunal diversity in Southeast Asia (Diesmos et al., 2002). It is home to a diverse group of amphibians and reptiles with a high percentage of endemism (Nuñeza and Galorio, 2015). The country houses 112 amphibian species, and 94 (83.9 %) of which are endemic (Diesmos et al., 2015).

Albeit the country's diverse record, the Eastern Mindanao Corridor (one of the Philippines largest remaining dipterocarp forest blocks) is experiencing diminishing forest cover due to timber extraction and mining (CEPF, 2001). Furthermore, studies within the corridor are scarce, especially among ecologically important habitats such as watersheds. Watersheds are critical habitats that sustains water-dependent organisms (Sedell et al., 2000) and provides water supply services to watershed populace. However, urbanization, land-use changes and intensive human activities threatened the integrity of this essential habitat (Shen et al.,2017). Calayagon watershed is one of these important watershed reserves found within the Caraga Region of Eastern Mindanao Corridor facing anthropogenic threats. It is also one of the major sub-watersheds of the Agusan River Basin experiencing high annual soil eroison rates (above tolerable levels) (ARB Integrated Water Resources Management Project, 2011). Reports on various human-induced activities such as forest conversion to agriculture, logging and insurgencies were recorded in the area.

As typical inhabitants of freshwater environments, anurans are first to suffer the consequences of environmental threats (Bishop et al., 2012). Due to their sensitivity they are regarded as biological indicators of pollution and are way more sensitive than other wildlife species (Hopkins, 2007; Simon et al., 2011). They are good representatives in studying freshwater habitats (such as watersheds) because of their high abundances (Burger and Snodgrass, 1998). Furthermore, knowledge of diet and resource partitioning is vital in understanding species' ecological niche (Piatti and Souza, 2011). Prey selection of anurans are important to understand food preferences (Almeria and Nuñeza, 2013), most especially for endemic anurans. There are no current studies conducted for feeding habits of anurans in the Philippines with studies limited to the works of Fabricante and Nuñeza (2012), Almeria and Nuñeza (2013) and in the invasive alien species by Solania et al., (2019), Apayor-Ynot et al., (2017) and Jabon et al., (2019). In order to understand the diversity of anurans, it is of prime importance to look into their food diets.

Currently, there are no known studies conducted in aforementioned key site. Thus, a study on the diversity and diet of anurans in Calayagon watershed was conducted. Data gathered in this study will help as a source of information on anurans' status and notes on their food preferences in Calayagon Watershed. This is also the first study to document the diet of the Philippine endemic *Fejervarya moodiei* and *F. vittigera*.

MATERIALS AND METHODS

Locale of the study and Establishment of Sampling Stationsa

Calayagon watershed is one of the primary sources of potable drinking water and irrigation for the surrounding community and in the nearby areas of Buenavista.

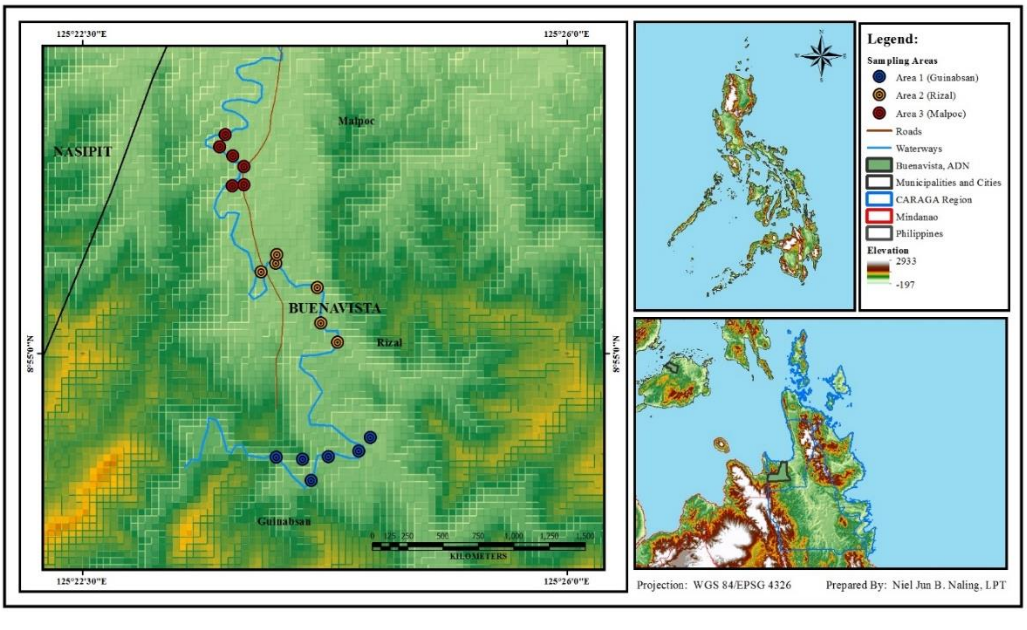

Figure 1: Map showing the Philippines and Northeastern Mindanao and the three sampling sites in Calayagon Watershed, Buenavista, Agusan del Norte, Philippines.

It was proclaimed a vital watershed for forest development and management through Memorandum Circular No.2008-04 by the Department of Environment and Natural Resources last 2008.

The study was conducted in the three localities of Calayagon watershed, Buenavista, Agusan del Norte from December to February 2019 with Wildlife Gratuitous Permit number R13-2019-03.

The three sampling sites surveyed were: Barangay Malpoc, Barangay Rizal and Barangay Guinabsan (Figure 1).

Six 100 x 10 meters transect line (Supsup et al., 2016) was laid in each sampling site, cutting across each barangay's different habitat types. Most of the riparian zones were characterized as cultivated areas with patches of secondary growth forest. Several anthropogenic activities were also observed in the area.

Anuran Survey and Identification

Extensive opportunistic field sampling in the established transect lines in each sampling site was conducted. The anurans' possible microhabitats were noted (riparians areas, cavities of rocks, leaf axils, under logs). The sampling activity involved five field workers searching for eight hours per day for 12 consecutive days. Collection of anurans was done in the morning (800 to 1200) and in the evening (1800 to 2200H) for a total of 480 man-hours.

The mark-recapture technique was done using water-based paint in between toes before it was released back to the same habitat (Mapi-ot et al., 2015). The identification of species was done through the works of Diesmos et al., (2015) and Sanguila et al., (2016). Identified species sample images were sent to Dr. Rafe Brown for verification and validation of the anurans captured.

Conservation and Distribution Status

The conservation status of anurans species were determined based on the IUCN Red List of Threatened Species 2018. Endemic and Non-endemic of amphibians were determined based on the existing journal of Diesmos et al., (2015) and Sanguila et al., (2016).

Diet Analysis

Only the species which had the most number of individuals in each sampling site were subjected to diet analysis. The standard number of individuals for diet analysis was 10 % of the population per species. Sizes and maturity were the same in all samples. The stomach was removed and dissected longitudinally, and contents were placed in 10 % formalin and preserved in 70 % ethanol. The stomach contents were spread in a petri dish and examined under a dissection microscope. All inside stomach contents were identified, based on their morphology, either to the order level or group, and the numbers of all prey types were recorded.

Each prey item's morphospecies was identified and classified according to four taxonomic classifications: Plant matters, insect group, inorganic materials (Almeria and Nuñeza, 2013; Solania et al., 2019), and other groups.

Frequency of occurrence (FOO) (Santos et al., 2004) and Degree of Food preference (DFP) (Braga, 1999) were quantified. In DFP, food was ranked into 4 (only one food item present in the stomach).

$$\text{FFO} = \frac{\text{Number of stomachs an item appeared}}{\text{Total number of stomachs with content}} \text{ x } 100$$

$$\text{DFP} = \frac{\text{Sum values given to the food item}}{\text{Total number of gut examined}}$$

Data Analysis

Paleontological Statistical Software Package (PAST) was used to analyze the biodiversity indices such as species abundance, richness, diversity, and evenness. Relative abundance (RA) was also computed by the abundance of species divided by the total abundance of all species.

RESULTS AND DISCUSSION

Anuran Species Composition

A total of 195 individuals, consisting of seven species from four families, were collected and identified from the three localities of Calayagon Watershed, Buenavista, Agusan del Norte (Table 1). The most richest family in terms of species composition is the Family Dicroglossidae with four species, which includes *Limnonectes magnus*, *Hoplobatrachus rugulosus*, *Fejervarya moodiei* and *Fejervarya vittigera*. The family represents one of the most ecologically diverse species-rich frogs (Frost, 2016). Only the cane toad, *Rhinella marina*, was recorded in the area to be from the Family Bufonidae. Cane toads generally have a higher abundance in disturbed areas than natural environments (Zug and Zug, 1979). These anuran species were commonly found in cultivated and agricultural regions. It is usually found in human-altered habitats and is visibly tolerant to disturbance. This anuran species could survive in higher disturbed areas and consume various food resources (Fredericksen and Fredericksen 2004; Solania et al., 2019).

Furthermore, *Kaloula pulchra* was the only species from the Family Microhylidae. This anuran family was mostly found in tropical and temperate regions, arid deserts, extremely wet rainforests, and almost everywhere (Van der Mejiden et al., 2007). Lastly, among the species from the Family Rhacophoridae, only *Polypedates leucomystax* was recorded.

In terms of conservation status, 72 % of the species recorded were Least Concern species because of their flexibility to environmental changes (IUCN, 2018). There is only one near-threatened species recorded which is the *L. magnus* (14 %). This anuran species can be found in undisturbed or minimally disturbed habitats and is highly threatened due to hunting for consumption (Sanguila et al., 2016). Lastly, *F. moodiei* is a Data deficient species (14 %) of the IUCN. Data Deficient species were likely to be more threatened with extinction than the other species fully assessed by the IUCN (Howard and Bickgford, 2014; Morais et al.,2013). Furthermore, four anuran species recorded were philippine endemic (57 %), two were invasive alien species (29 %), and one was non-endemic species (14 %).

Amphibian surveys conducted in nearby watersheds such as in Bega watershed in Agusan del Sur recorded 13 species and 54 % of which are Philippine endemic species (Calo and Nuñeza, 2015) while 20 amphibian species were recorded from Taguibo watershed in Butuan City, Agusan del Norte (Sanguila et al., 2020). There are less number of amphibian species recorded in the riparian zone of Calayagon watershed compared to nearby watersheds. The low counts could be due to agriculture and anthropogenic activities along the riparian banks. There were also no forested areas along the riparian zone with forested areas located away from the banks. Studies showed that secondary growth forests harbors higher species richness compared to mixed-use agricultural areas (Supsup et al.,2020).

Among all the species recorded, *F. vittigera* was the most abundant (44.66 %), probably due to the abundance of its preferred aquatic microhabitat; river, streams, and ricefields. *P. leucomystax*, on the other hand was the least recorded species (1.54 %).

This species was known to inhabit banana plantations (Sanguila et al., 2016; Solania and Gamalinda, 2018). Since the watershed's riparian zone is mostly characterized by the cultivation of Falcata trees, the absence of its preferred microhabitat may have caused its low abundance.

Anuran Biodiversity Indices

The Biodiversity Indices in all sampling stations was shown in Table 2. Barangay Guinabsan was recorded to be the highest in terms of species richness, abundance (tied with barangay Rizal), and diversity (S=8, N=70, H'=1.58, respectively). The area was observed to hold the most water, favoring the abundance of this water-dependent species.

Table 1: Species Composition, Conservation Status, Endemicity, and Abundance of anurans recorded from the three localities of Calayagon Watershed of Buenavista, Agusan del Norte during the sampling period.

Family	Taxon	Common Name	Conservation Status*	Endemicity**	Abundance (Relative Abundance%)
Bufonidae	*Rhinella marina*	Giant Cane toad	LC	IAS	41 (21.02)
Dicroglossidae	*Fejervarya moodiei*	Crab eating Frog	DD	PE	44 (22.56)
	Fejervarya vittigera	Luzon-wart frog	LC	PE	87 (44.62)
	Hoplobatrachus rugulosus	Bullfrog	LC	IAS	9 (4.61)
	Limnonectes magnus	Giant Philippine Frog	NT	PE	4 (2.05)
Microhylidae	*Kaloula pulchra*	Banded bullfrog	LC	PE	7 (3.59)
Rhacophoridae	*Polypedates leucomystax*	Four lined tree frog	LC	NE	3 (1.54)

**Conservation Status; LC=Least concern, NT=Near Threatened, DD=Data Deficient. **Endemicity; PE=Philipine Endemic, NE=Non-endemic, IAS=Invasive Alien Species*

Table 2: The species richness, diversity, evenness, and dominance of anurans were found in the sampling sites during the sampling period.

Sampling Site	Species Richness (S)	Abundance (N)	Species Diversity (H')	Dominance (D)	Evenness (E)
Guinabsan	6	70	1.58	0.23	0.81
Rizal	4	70	0.99	0.46	0.67
Malpoc	4	55	1.22	0.34	0.85
Total	**7**	**195**	**1.43**	**0.30**	**0.60**

The presence of various habitats such as cultivated areas and secondary-growth forests along the riparian zone may also have contributed to the abundance of species in the area.

The overall species diversity index value of the area was H'=1.43. The high diversity could be due to the abundance of foraging sites and breeding sites. Species evenness was high (E=0.60) while species dominance was low (D=0.30). High species evenness implied an almost equal number of individuals per species, with no single species dominating the area.

However, several anthropogenic activities observed in the watershed may affect amphibians' diversity in the area. Urbanization is known to affect aquatic ecosystems (Riley et al., 2005) and decrease anuran species diversity (McKinney, 2008). Effects of urbanization such as roads and changes in soil moisture significantly influence the presence of amphibians (Aryal et al.,2020). Urbanization has altered the stream habitats in watersheds leading to negative effects on native amphibian diversity and abundance (Riley et al.,2005). Modifying the hydrology of streams due to anthropogenic activities can disrupt local vegetation and indirectly detrimental to amphibian species (Canessa and Parris, 2013). Small and large-scale logging and cutting of trees for human settlements could affect the microhabitats of frogs. Changes in the landscape are known to have a severe impact on anurans' distribution and population by physically altering aquatic and terrestrial environments (Wilbur, 1980).

Diet Composition of R. marina, F. vittigera and F. moodiei

Diet analysis is an effective way to identify organisms that are being consumed by another organism and therefore provide inference into the dynamics of ecological balance in a habitat (Santos et al., 2004; Simon and Toft, 1991). It will also provide essential information in order to develop conservation strategies (Anderson, 2001). The anuran species that underwent diet analysis were *Rhinella marina* from Barangay Guinabsan (station 1), *F. moodei* from Barangay Rizal (station 2), and *F. vittigera* from Barangay Malpoc (station 3). Figure 2-5 shows the prey items recorded in all the anuran species examined.

Plant matters which includes grains, seeds, and plant debris where found in the stomach of the anuran species examined (Figure 2A-F). Leaf debris (Figure 2G) and twigs (Figure 2H) were also collected. Remnants of insects from orders Coleoptera, Hymenoptera, Orthoptera, Odonata, and some Insect debris were recorded (Figure 3). Stones and pebbles were classified as Inorganic matters (Figure 4), and remnants of Gastropod, Millopod, and Amorphous substances were classified as Other Groups (Figure 5).

Anurans are thought to be opportunistic feeders that prey on any smaller organisms that crossed their line of vision (Browne, 2009; Toft, 1995). Amphibians are previously known to be insectivores/carnivorous, however, new records suggest they are generalist species (Ahlm, 2015). Essential nutrients of amphibians includes the right amount of calcium/phosphorous ratio, lipid/protein ratio and micronutrients (Vitamins D3, Vitamin A and Carotenoid). These nutritional requirements along with species feeding mode influence the choice of feeding regime. The presence of prey items equates to good body condition and health which are important for successful amphibian reproduction (Browne, 2009).

Table 3 shows the recorded prey items found in the gut of the most abundant anurans species in each sampling site. The most abundant prey items in the gut of *R. marina* were plant matters followed by remnants of the insect body. In terms of frequency of food occurrence, plant debris, grains, seeds, remnants of beetles, ants, other insect debris, and millopods were constantly recorded in all samples examined. The results showed that plant debris was noted to be preferential in terms of the degree of food preference, and the rest were secondary.

According to Isaacs and Hoyos (2010), Solania et al., (2019), and Jabon et al., (2019) R. marina is a generalist species, consuming all types of food. This was also proven in the study because of the presence of other prey items rather than insects alone. R. marina can adjust in habitats with high anthropogenic activities and mostly degraded habitats, thus able to consume available food items (Solania et al., 2019).

According to Apayor-Ynot et al., (2017) gut contents, of R. marina from Davao City recorded insect matters such as Blattodea, Orthoptera, Chilopoda, Hymenoptera, Coleoptera, Hemiptera, Diplopoda, Annelida, Isoptera, plant matters such as leaves, twigs, seeds, woods and other matters such as pebbles and thread. The study of Solania et al.,(2019) also showed that plant debris dominated the gut contents of R. marina from Butuan City.

The most abundant prey item in the gut of *F. moodiei* were plant matters and insect orders followed by insects' remnants. In terms of frequency of food occurrence, plant debris, grain, seeds, remnants of ants, bees, dragonflies, insect debris and amorphous substances were constantly recorded in all samples examined.

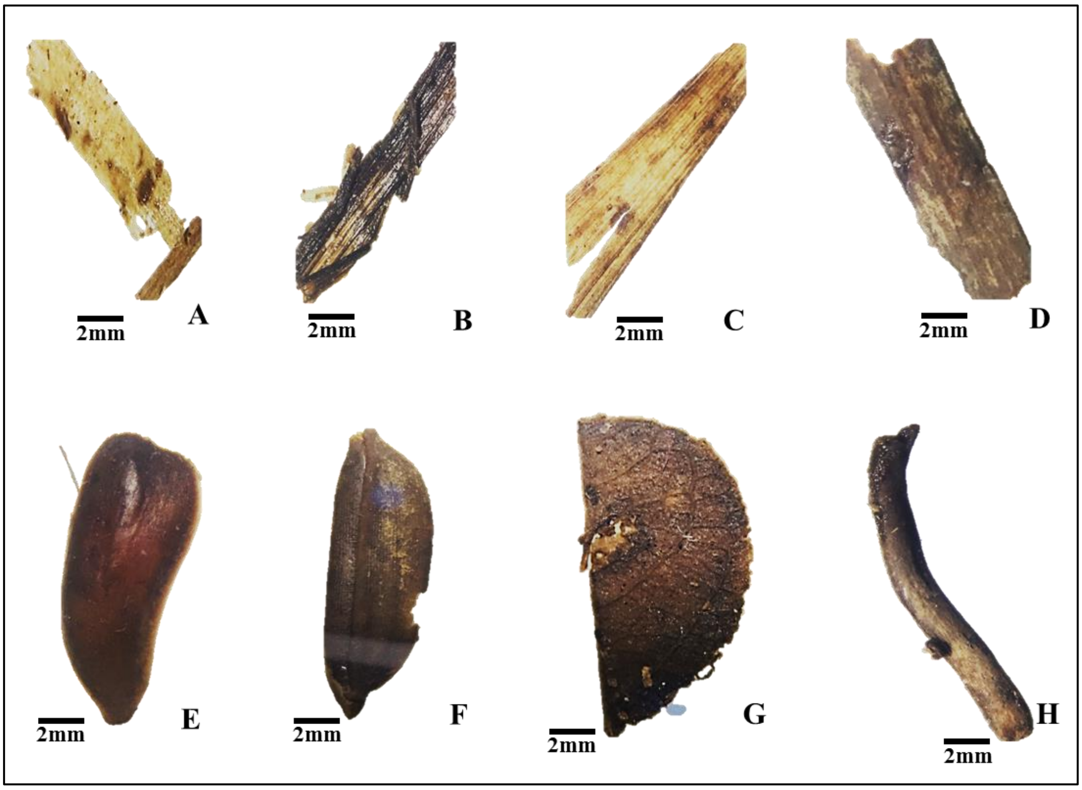

Figure 2: Plant matters. A-D. Plant debris, E. seed, F. grain, G. Leaf and H. Twig.

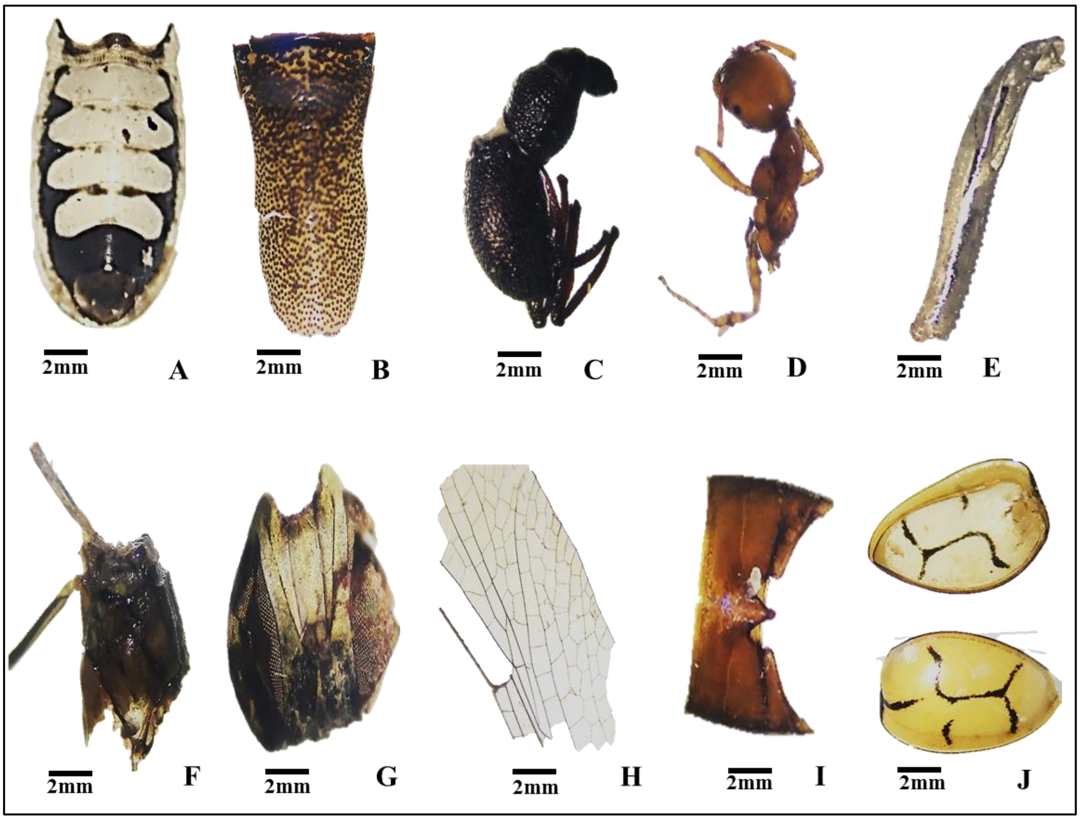

Figure 3: Remnants of Insect Orders. A-B. and J. Coleoptera, C-D. Hymenoptera, E-H. Odonata, and I. Orthoptera.

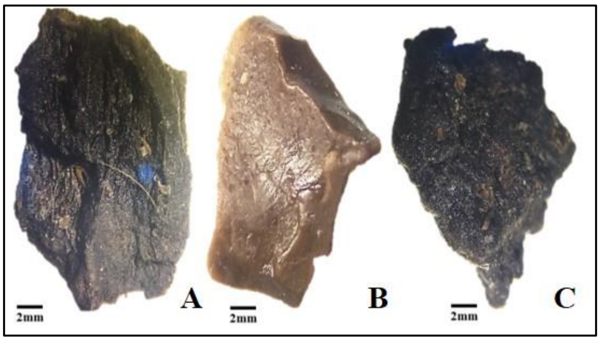

Figure 4: Inorganic Matters. A-C Stones and Pebbles.

Figure 5: Other Groups. A. Millopod remnant and B. Gastropod remnant.

Table 3: Percentage composition (in %) of Food items in the stomach of *Rhinella marina, Fejervarya moodei* and *Fejervarya vittigera* recorded in each sampling sites during the study period.

	Rhinella marina Brgy. Guinabsan			*Fejervarya moodei* Brgy. Rizal			*Fejervarya vittigera* Brgy. Malpoc		
Prey Item	**# of prey items**	**Frequency of food Occurrence**	**Degree of food Preference**	**# of prey items**	**Frequency of food Occurrence**	**Degree of food Preference**	**# of prey items**	**Frequency of food Occurrence**	**Degree of food Preference**
A. Plant Matters									
1. Plant Debris	48	100^{c}	3^{p}	23	100^{c}	2^{s}	72	100^{c}	3^{p}
2. Grain	9	100^{c}	1^{s}	5	25^{s}	1^{s}	4	66.67^{c}	0.99^{o}
3. Seeds	32	100^{c}	2^{s}	2	25^{s}	1^{s}	0	0	0
B. Insect Orders									
1. Coleoptera	4	100^{c}	1^{s}	0	0	0	0	0	0
2. Hymenoptera	36	100^{c}	2^{s}	2	25^{s}	1^{s}	1	33.33^{s}	0.99^{o}
3. Orthoptera	2	50^{c}	1^{s}	0	0	0	0	0	0
4. Odonata	0	0	0	11	25^{s}	0.25^{o}	0	0	0
5. Insect Debris	18	100^{c}	1^{s}	29	100^{c}	3^{p}	28	100^{c}	1.98^{s}
C. Inorganic Matters									
1. Stone	2	50^{c}	1^{s}	0	0	0	0	0	0
2. Pebbles	0	0	0	0	0	0	0	0	0
D. Other Groups									
1. Gastropoda (snail)	1	50^{c}	1^{s}	0	0	0	0	0	0
2. Millopoda	5	100^{c}	1^{s}	0	0	0	0	0	0
Amorphous	1	50^{c}	1^{s}	9	75^{c}	1^{s}	4	33.3^{s}	0.99^{o}

** Frequency of Food Occurrence: C= constant (>50 %); S= secondary (25-50 %); A= accidental (<25 %). ** 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) (Santos et al., 2004).*

In terms of degree of food preference, the results showed that insect debris was noted to be preferential and the insect order Odonata were noted to be occasional while the rest were secondary.

The most abundant prey item in the gut of *F. vittigera* was plant debris and insect orders. In terms of frequency of food occurrence, plant debris, grain, remnants of ants, bees, insect debris and amorphous substances were constantly recorded in all samples examined. In terms of food preference, grain, hymenoptera, and amorphous substance were noted to be occasional, plant debris was noted as preferential, and insect debris as secondary. According to the study of Fabricante and Nuneza (2012) diet compositions found in *L. magnus* (a species from the same family as *F. vittigera* and *F. moodiei*) were dominated by plant matters and invertebrates such as Crustacea, Arachnida, Blattodea, Coleoptera, Diptera, Hymenoptera, Odonata, and Orthoptera.

Graminae seeds and undigested dicotyledon leaves, including plant debris, were also observed. Food availability in the area might be the reason for the abundance of *F. vittigera* and *F. moodiei*.

The ingestion of plant debris may be accidental when anurans preyed on insects perching on leaves. Plant debris was also a constant food group for some frog species such as *Hyla albomarginata, Hyla cf. Branneri, Hyla inuta, Phyllomedusa aff. hypochondrialis, Leptodactylus natalensis* and *Physalaemus cuvieri* (Santos et al.,2004). Plant debris of *R. marina* and *F. vittigera* recorded as preferential, in terms of food preference may be inferred as accidental ingestion, same as the stone and pebbles. However, plant matters found in frogs' gut may help eliminate intestinal parasites, provide roughage to assist in grinding and processing the invertebrates, or provide nutrients and additional water source (Fabricante and Nuneza, 2012). In addition, plant consumption contributes to the understanding of behavioral patterns on amphibians. The presence of plant matters in the gut indicates that vegetation may is not only used as a reproductive site, but it is also for foraging territory (Almeria and Nuneza, 2013).

CONCLUSIONS

This study was the first to record the amphibian species along the riparian zone of Calayagon Watershed. The preliminary opportunistic survey recorded a near-threatened and data deficient species with a high overall diversity value implying the area’s conservation importance. Diet analysis was also conducted for *R. marina, F. moodei* and *F. vittigera* wherein insects and plant debris were the most abundant and preferred food items of the species. The study will served as baseline information for the food preferences of *F. moodei* and *F. vittigera* and additional knowledge for the food preferences of *R. marina.*

The study highly recommends strict policies to help lessen the anthropogenic disturbances in all areas of Calayagon Watershed to sustain and provide more rich and healthy waters to agricultural areas and nearby communities and protect and preserve the anuran species. Lastly, surveys on unexplored forested areas and interviewing locals within the area are also encouraged.

ETHICAL APPROVAL OF STUDY

The study secured DENR Gratuitous permit for the conduct of the study with Gratuitous Permit number R13-2019-03.

CONFLICT OF INTEREST

The authors declare no known conflict of interest.

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