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Diet Composition and Endoparasitic Load of the Cane Toad, *Rhinella marina* (Linnaeus 1758) from Selected Localities of Butuan City, Philippines

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A B S T R A C T

Rhinella marina is an introduced, invasive, and generalist species that prefer open and disturbed habitats. The study was conducted in the four non-forested barangays of Butuan City between February-May, 2018, and 40 samples of *R. marina* were captured for diet and endoparasite analyses. Following gut content analysis, the stomach, intestine, and liver were excised. The plant debris dominated the food items of the samples collected in Barangay Cabcabon (82.50%), Libertad (57.50%), and Sumilihon (67.50%) while stones, pebbles, and plastics yielded 62.50% in the stomach contents of samples from Barangay Anticala. Plant matters (65%) were the overall most representative component, while insect prey was the least recorded food item (4%). The species flexibility towards food items makes it qualify to be an omnivorous species. The endoparasite examination recorded 34 individuals of helminths, 17 nematodes, and 17 trematodes from 14 infected hosts with an overall prevalence rate of 35%. It was noted that no two types of parasites co-exist with one another. Using the Spearman non-parametric correlation, it was recorded that there was no significant positive correlation between the diet and the parasite load in the host (p-value = 0.734; R^2 = -0.55). The results suggest that the generalist omnivore behavior of *R. marina* could out-compete native and endemic anuran fauna in terms of food and space. The species could also act as a reservoir and could spread the endoparasites to the native amphibians within the habitat.
Keywords: *Food items, omnivore, endoparasites, invasiveness*

1 Introduction

The cane toad, *Rhinella marina* is a widely known introduced anuran species native from South Texas to Central Amazonia. However, due to various processes that occur naturally, environmental factors, and as well as human activities, this introduced taxa have flourished and thrived to places far distant from their native ranges. This species then spread and set-up populations in Central United States, Australia, Philippines, Japan, Taiwan, Papua New Guinea, and some islands across the Caribbean and the Pacific (Acevedo et al. 2016). Exotic species are more of concern because once it had established populations, it could negatively impact native biodiversity in the area (IUCN, 2000).

Aside from being an introduced species, *R. marina* was recognized by the Invasive Species Specialist Group (ISSG) as an invasive species. The species are likely to consume insects such as ants, caterpillars, grasshoppers, millipedes, and other small vertebrate animals, including lizards. *R. marina* was reported to consume human feces, rotting garbage, and cigar butt, proving the generalist characteristic of the species (ISSG, 2008). It may interact with native anurans and might bring severe ecological impacts (Pizzatto & Shine, 2011). Potentially, these heighten more catastrophic effects, for it acts as a reservoir of diseases towards native taxa (Poulin & Mouillot, 2003). The *R. marina* species can also spread and disseminate pathogens and parasites to the native fauna. The common endoparasites of amphibians include nematodes,

trematodes, and acanthocephalans (Rahman & Shakinah, 2014).

There are limited information in the diet (Ates et al. 2007) and endoparasites of amphibians in the Philippines. This study was first to present records on the diet and endoparasite of the invasive alien species, *R. marina* from the selected non-forests barangays of Butuan City. The diet and endoparasite analysis may provide inference on the dynamics of the ecological tolerance of the species.

2 Materials and Methods

Collection Sites

The toad samples were obtained from the four selected barangays of Butuan City, Agusan del Norte, Philippines. The collection sites were non-forested habitats since this species commonly thrives along with anthropogenic settlements (Sanguila et al., 2016). The collection sites of Station 1 (Barangay Anticala) is characterized by canals and waterways, station 2 (Barangay Cabcabon) is dominated by farmlands and grassland habitats while station 3 (Barangay Libertad) and 4 (Barangay Sumilihon) were residential areas. Ten individuals of *R. marina* in each station were collected manually through the handpicking method for a total of 40 individuals.

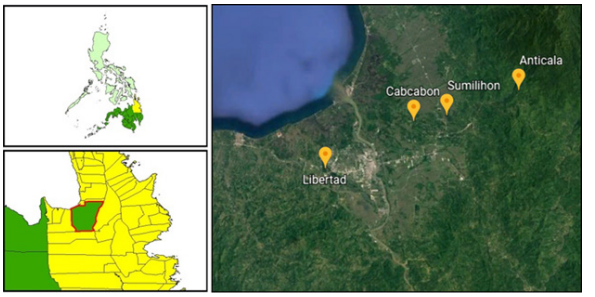

Figure 1. Map of the Philippines and Butuan City (inset) and the locale of the collection sites; Barangays Anticala, Cabcabon, Libertad, and Sumilihon

Diet Composition and Diet Analyses

External abnormalities of *R. marina* were examined before dissection. The whole digestive tract of each specimen, starting from the esophageal area to the rectum was removed, sectioned, and examined. For the diet examination, each section of the digestive tract was carefully openned. Stored food and fecal material were collected, analyzed, and identified under a dissecting microscope, and the percentage occurrence of prey items was calculated. To assess the morphospecies in the diet of *Rhinella marina,* each prey item was determined and classified according to their taxonomic classification. Prey items were categorized into three food groups: plant matters, insect groups, and inorganic materials (Almeria & Nuñeza, 2013).

The frequency of occurrence (FOO) was quantified using the formula of Santos et al. (2004), while the Degree of Food preference (DFP) was calculated using the index of Braga (1999). In DFP, food was ranked between 1-4; '4' when only one food item is present in the stomach, '3' was assigned to the most abundant food group, '2' to the second most abundant and '1' to the least abundant.

FOO = (Number of stomachs an item appeared)/(Total number of stomachs with content) x 100
DFP = (Sum values given to the food item)/(Total number of guts examined)

Endoparasite Examination

The stomach, intestine, and liver were recovered and examined for endoparasites using the dissecting microscope. The prevalence of endoparasites and abundance were computed. The index of infection was calculated using the formula of Bhure et al., 2007:

Prevalence = (Total Number of Infected Frog)/(Total Number of Frog Host Examined) x 100
Abundance = (Total Number of Parasites Recovered)/(Total Number of Frog Host Examined)
Index of Infection = (Number of Host collected Infected x Number of Parasites)/(Total Number of Host Examined)

The identification of the endoparasites was based on the gross external morphology following the

descriptions of Amherst College (2008).

Correlation between Diet and Endoparasite of Rhinella marina

The Spearman Non-parametric correlation was used to determine the relationship between the diet composition and the presence of parasites across all samples examined. The parasites harbored by individual *R. marina* were tallied as well as the diets present in the organism.

3 Results and Discussion

Diet Composition of Rhinella marina

Ten out of the 40 toad samples (25%) were empty stomachs, and the remaining 30 samples contained dietary information (75%). Biavati et al. (2004) hypothesized that amphibian samples with empty stomachs could be due to seasonality and patchy distribution of food. It could also be due to the sex of specimens since males tend to give more time in search of a mate and not for food. There were 13 types of food items recovered in all 30 toad samples. Plant matters consist of plant debris such as leaves and twigs, grains, and seeds (Figure 2). Insects prey items were identified as belonging to orders Hymenoptera, Coleoptera, Orthoptera, and Blattodea (Figure 3). The hymenopterans were mostly black and red ants, coleopterans excised were beetles, several remnants of grasshoppers (Orthoptera), and intermediate parts of cockroaches (Blattodea) were recorded. The inorganic materials include plastics (size range 2-5cm), hair, pebbles/ stones, soil, electrical tape (5cm), and feathers (2cm) (Figure 4). The data was first to record plastics, tape, and feather in the diet content of *R. marina*, although it was perceived as accidental.

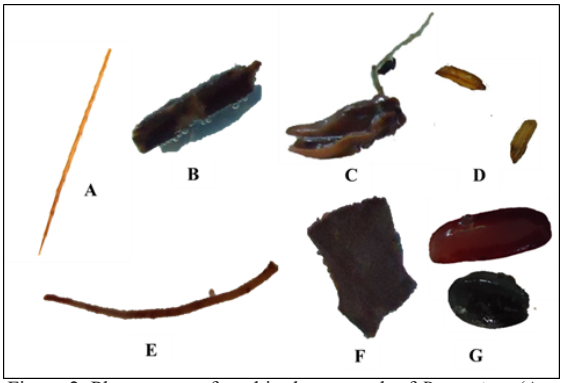

Figure 2. Plant matters found in the stomach of *R. marina*: (A, B & E) plant debris, (C) coconut debris, (D) grains, (F) dry leaf and (G) seeds.

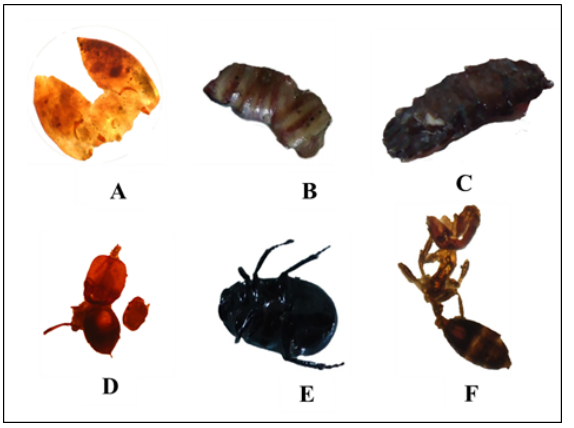

Figure 3. Insect prey items of *R. marina*: (A) Coleopteran remnant, (B) unidentified larvae, (C) blattodea fragments, (D) hymenopteran, (E) coleopteran (beetle), (F) hymenopteran (ant) at 10x magnification.

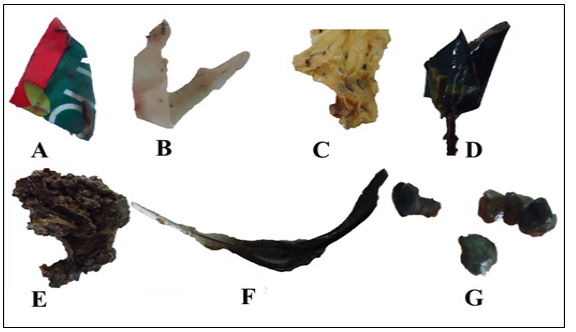

Figure 4. Inorganic materials as food items of *R. marina*: (A-C) plastic traces, (D) electrical tape, (E) soil, (F) feather, and (G) pebbles.

Percentage Occurrence of Food Diets

Inorganic prey items, which include stones, pebbles, and plastics, dominate the food preferences of *R. marina* from Barangay Anticala (62.50%). High inorganic materials were recorded since frogs obtained from this Barangay were collected near canals and water channels. The diet of *R. marina* from Barangay Cabcabon was comprised of plant debris (83.5%) and inorganic prey items (16.5%). Insects were not recorded among the ten samples from this barangay. Plant debris was the dominant

food type of this toad, for it was acquired along with leaf litters of the barangay far from the local community. Plant debris also dominates the gastrointestinal tract of samples from Barangay Libertad (65.61%). Toads from this barangay were collected near agricultural lands and residential sites, possibly giving the high percentage of plant debris and inorganic prey items in the food component. Furthermore, plant materials were also the most seen food type in samples from Barangay Sumilihon followed by inorganic materials. Insect ingestion was not seen among the frog samples in this barangay (Table 1).

Table 1. Percentage composition (%) of Food items in the stomach of *Rhinella marina* recorded in each collection site during the study period.

Prey Item	Percentage Composition (%)				Degree of Food Preference*	Frequency of Occurrence (%)**
	Barangay Anticala	Barangay Cabcabon	Barangay Libertad	Barangay Sumilihon		
A. Plant Matters						
1. Plant Debris	34.17	82.50	57.50	67.50	1.25 ^{S}	53.33 ^{C}
2. Grain	-	-	8.11	10.84	0.20 ^{O}	13.00 ^{A}
3. Seeds	-	1.00	-	-	0.075 ^{O}	3.33 ^{A}
B. Insect Orders						
1.Hymenoptera	1.11	-	1.30	-	0.2 ^{O}	10.00 ^{A}
2. Coleoptera	1.11	-	1.30	-	0.10 ^{O}	6.66 ^{A}
3. Orthoptera	-	-	1.30	-	0.025 ^{o}	3.33 ^{A}
4. Blattodea	-	-	7.00	-	0.10 ^{O}	6.66 ^{A}
5. Insect fragments	1.11		1.30		0.25 ^{O}	10.00 ^{A}
C. Inorganic Materials						
1. Plastic	3.33	-	7.00	5.41	0.125 ^{O}	13.00 ^{A}
2. Hair	-	3.75	3.27	-	0.125 ^{O}	6.66 ^{A}
3. Pebbles/stones	59.17	9.00	2.60	10.84	1.075 ^{S}	43.33 ^{S}
4. Soil	-	-	8.00	-	0.075 ^{O}	3.33 ^{A}
5. Electrical tape	-	-	-	5.41	0.025 ^{O}	3.33 ^{A}
6. Feathers	-	3.75	1.30	-	0.15 ^{O}	6.66 ^{A}

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

A high percentage composition of plant debris (65%) was recorded in all samples (Figure 5). The ingestion of plant matters in anurans is frequent and may not be intentional (Maragno & Souza, 2011). Plant matters were indirect food items for *R. marina* since this species is a carnivore (Ynot et al., 2017). Amphibians may have ingested plant matters for the digestion of arthropod exoskeleton (Anderson et al., 1999) or could be accidental while foraging for invertebrates (Almeria & Nuñeza, 2013). Plant matters could serve as a source of water to prevent dehydration (Batista et al., 2011). Amphibian herbivory incidence was reported by Isaacs and Hoyos (2010), Lajmanovich (1994), and Santos et al. (2004) in Colombian Coffee regions, and Almeria and Nuñeza (2013) in Agusan Marsh.

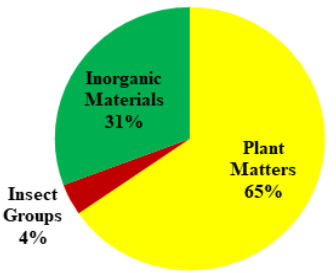

Figure 5. Overall percentage composition of food items of *R. marina* in Butuan City, Agusan del Norte, Philippines

In terms of inorganic food group, it ranked second in occurrence (31%). Ynot et al. (2017) also recorded pebbles and threads in the gut

of *R. marina* from Davao City. Stones were also recorded in the study of Kidera et al. (2008) from *R. marina* samples from Southern Ryukyus, Japan. Several literatures also support this behavior, such as dog and cat food in the stomach of *R. marina* from Southern Florida (Krakaguer, 1968) and rotting garbage and cigarette butt (Lever, 2001). Soil particulates (8%) were also recovered in the gut of samples from Barangay Libertad. *R. marina* samples from Agusan Marsh also yielded 20% soil particulates in their diet (Almeria & Nuñeza, 2013).

In the present study, insect groups were the least recorded prey item (4%). Hymenopterans and Coleopterans were the most frequently encountered insect food items. Formicidae (ants) were previously reported to be the most common food item in the stomachs of *K. conjuncta*, *P. leucomystax, O. laevis, F. cancrivora,* and *P. acutirostris* (Ates et al., 2007). According to Alcala and Brown (1998), most of the Philippine anurans prefer invertebrates. Almeria and Nuñeza (2013), and Ynot et al. (2017) recorded invertebrates as the chief component in the diet of *R. marina* from Davao City. The low abundance of insect prey in the collection sites could be the reason behind the low insect occurrence in the stomachs of the samples. However, there were recorded insect fragments that were partially digested and could no longer be identified into taxonomic orders, that could lead to the underestimation of insects as prey items (Santos et al., 2004).

The prey items of anurans can reflect the feeding strategy used by the species: sit-and-wait foragers consumed few food items that are large and mobile, and opportunistic searchers consume smaller food items in more significant quantities (Solé & Rödder 2010). According to the data gathered, the distinct prey items found in the diet composition of *R. marina* can be characterized as opportunistic searchers since there were a large amount of smaller food items. Biavatti et al. (2004) reported a relationship between predators and prey size or prey number. The stomach contents and differences in prey items suggest differences in habitat occupation.

In the absence of insect prey, *R. marina* could still persist because of its opportunistic behavior and generalist feeding habit. Previous reports of the feeding behavior of *R. marina* also coincides with the results such as in, Almeria and Nuñeza (2013), Isaacs and Hoyos (2010), Krakaguer (1968), Lever (2001), and Ynot et al. (2017). The results suggest that since it is a generalist, *R. marina* can be perceived as an omnivorous species. This behavior could impose competitive exclusion pressure on native amphibian fauna (Kidera et al., 2008).

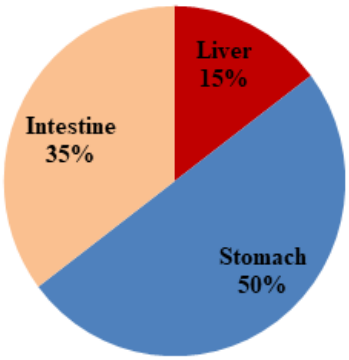

Figure 6. Occurrence of parasites in each selected digestive organ of the examined *R. marina*

Degree of Food Preference and Frequency of Occurrence

Frequency of Occurrence (FOO) recorded plant debris to be a constantly occurring food item in the stomachs examined. Pebbles and rocks were secondary occurring food item, and the other food items were considered to be accidentally consumed by *R. marina*. In terms of the Degree of Food Preference (DFP), both plant debris and pebbles were considered secondary preferential food items while the others were regarded as occasionally preferential food (Table 1).

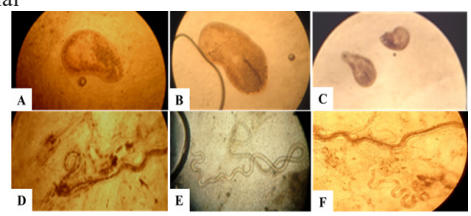

Figure 7. Endoparasites of *R. marina;* A-C trematodes and D-F nematodes (10x magnification)

Endoparasite Analysis of Rhinella marina

Endoparasite examination recorded 14 infected hosts out of 40 toad samples. The overall prevalence of infection was 35%.The stomach is frequently the most affected digestive organ (50%) followed by the intestine (35%) and lastly the liver (15%) (Figure 6). The stomach is frequently the most infected digestive organ (50%) followed by the intestine (35%) and the liver (15%). This data coincides with the results of Rahmn et al. (2008) that nematodes dominated the stomach and intestine of *Bufo melanosticus*. Silva et

al. (2014) reported the larva of the helminth, *Ortleppascaris* sp., which infects the liver parenchyma of *R. marina*. Trematodes were also recorded in the liver of the present study, although not identified to the species level. The *R. marina* is known to inhabit even highly polluted waterways and highly degraded habitats. This behavior and its generalist nature could have paved the way for the ingestion of these parasites.

Table 2 shows the abundance of the accumulated parasites among ten frogs in every sampling site. Barangay Anticala and Libertad both had the least number of infected toads (N=2), four toads were infected from Barangay Sumilihon while Barangay Cabcabon had the most number of affected toads (N=6). A total of 34 helminths were recovered from the 40 examined specimens. Helminths such as nematodes and trematodes were identified (Figure 7).

Table 2. Endoparasite classification from samples in each barangay during the study period.

Sampling Sites	Number of Infected Toads	Number of Individual Endoparasites	
		Nematodes	Trematodes
Barangay Anticala	2	3	0
Barangay Cabcabon	6	9	3
Barangay Libertad	2	1	5
Barangay Sumilihon	4	4	9
Total Number of Parasite Recovered		17	17

Of the 37 parasite individuals, 17 were nematodes and 17 trematodes. Barangay Cabcabon had the most number of nematodes, and Barangay Sumilihon is highest in terms of trematode abundance. The results conformed with Rahman and Shakinah (2014), wherein nematodes and trematodes topped the list of parasites found. Nematodes exceedingly thrive in a wide variety of habitats such as in soil, marine, and freshwater bodies or decomposing matter (Roberts & Janovy, 2005) making organisms living directly above the leaf litter vulnerable to infections. Aho (1990) recorded that nematodes were the most dominant helminth in *R. marina*.

Nematodes, trematodes, and acanthocephalans were the helminths found on the amphibian, *Ptychadena mascareniensis* from African countries (Imasuen & Aisien, 2012). The parasitic fauna of *R. marina* from Tarapoto, Peru recorded one species of cestode and six species of nematodes. The endoparasites were composed largely of nematodes indicating that amphibians are associated to the terrestrial environment (Toledo et al., 2017). Bolek and Coggins (2000) recorded 92% nematodes, 6% cestodes, and 2% trematodes in the gut of *R. marina* from Waukesha Country, Wisconsin, USA. Nematodes could infect amphibians through skin penetration (Bolek & Coggins, 2000) or by ingesting infected larvae (Anderson, 2000).

Although a terrestrial species, *R. marina* also spend time in areas closer to permanent water (Novelo et al. 2017) thus, also acquiring infected arthropods. Trematode cyst infection could cause limb deformities in amphibians (Stopper et al. 2002). The movement of the cane toad *R. marina* between the terrestrial to aquatic habitats over its lifecycle favors parasite establishment (Novelo et al. 2017). Also, the period of reproduction (time spent in the terrestrial and aquatic habitats), according to Aho (1990), is a determinant of the abundance of parasites in amphibians.

The number of infected *R. marina* with nematodes is higher compared to that of the trematodes. Nematodes were highest in terms of prevalence rate (22.5%) and index of infection (3.825). The overall prevalence rate (35%) is low. However, since this species is a colonizer, this species could also potentially act as a reservoir of the parasite. They could spread the endoparasites to native amphibians through ingestion of fecal matter with infected larvae or skin penetration.

Novelo et al. (2017) concluded that there is a positive correlation between the abundance of parasites and host size. Since *R. marina* samples were adult toads, its capability to host parasites is not surprising. It was noted that there is only one class of parasite recorded in each host. Hence, no two classes of parasites co-exist with one another (when nematodes are present in the gut, no trematodes were recorded and vice versa).

Correlation Analysis between the Diet and Endoparasites of R. marina

The correlation of the diet and endoparasite of the 40 sample specimen was determined to be not significant (p value= 0.734). The diet of *R. marina* shows a moderate, negative correlation (R^2 = -0.55) to the presence of parasites in the infected host. Although predation was a necessary part of the transmission of the parasite in the system, the results show no significant correlation. The nematodes in the study of Akani et al. (2011), were found to be "monoxenous" and do not require an intermediate host since the infection was acquired through skin penetration. The diet was not integral to the presence of the parasite in the study. However, unlike nematodes, trematodes and cestodes require diet for it to be present in the host (Anderson, 2000).

According to Novelo et al., (2017), feeding habit is a minor source of parasites. Examinations of *R. marina* revealed that most samples with food items in the stomachs do not have parasites, and samples with endoparasites usually do not have food contents. However, there is a significant positive correlation between host body size and parasite richness (Hamann et al. 2012; Novelo et al. 2017). Therefore, endoparasites might be more related to host size than to the diet.

4 Conclusion

The gut content analysis revealed that *R. marina* is an omnivore species. High percentage composition of plant matter and inorganic materials were observed. The preferred food item of *R. marina*, which are insects, comprised only a small percentage of its recovered diet. Nonetheless, several unidentifiable insect fragments were seen in the gut. The low insect diet could have been due to a possible low insect population in the collection site. An alarming inference that insects in the non-forested habitats in Butuan City could have been declining due to anthropogenic expansion and industrial disturbances.

The generalist nature of *R. marina* and its ability to thrive even in degraded and polluted habitats could have been an entry point of parasites. The stomach was the most infected part of the digestive tract. A negative correlation between food diet and endoparasites was also noted and that no parasite co-existed with other parasites in one host. Further studies on dietary contents across seasons are recommended to obtain the diet pattern of *R. marina*. Identification of recorded endoparasites to species level will be the future direction of the study.

5 Acknowledgment

The researchers would like to thank the local barangay councils of Anticala, Cabcabon, Libertad, and Sumilihon for allowing the researchers to conduct the study in their area; the Biology laboratory personnel of Caraga State Univeristy for the assistance during the dissection process; and Niel Jun B. Naling for lay-outing the map of the study area.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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