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Diet Composition of *Rhinella marina* in the Mining Area of Gango, Libona, Bukidnon, Philippines

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Abstract

The diet of frogs is an important indication of the health of the environment. This study aimed to determine the gut content of 60 samples of *Rhinella marina* collected between the two sampling points in Gango, Libona, Bukidnon. Gut contents were identified and classified according to the nearest taxon. The most preferred food found in the two sampling sites was ants (31.51%) as the most abundant prey items in the gut of *R. marina*. Other prey items are considered to be ingested occasionally. It is also reported that soil, plant, rocks and solid wastes were ingested by the toad. Thus, *R. marina* is a generalist species and feeds on a wide variety of food items available in the habitat. It is recommended that further studies should be conducted on the diet of this frog to assess the effect of mining activities.

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Introduction

The Philippines is a major center of amphibian diversity and endemism (Diesmos *et al.,* 2006). Amphibians are commonly known to be one of the most diverse groups of species that are endemic high throughout the Philippine archipelago (Nuñeza *et al.,* 2015). To date, there are 80 known species of frogs and 92 species of reptiles in the Philippines, of which eight species of frogs are native to Mindanao Island and 60 of its species are limited to the Philippine archipelago (Relox *et al.,* 2011).

In recent centuries, as rapid human exploitation occurred, habitat loss, alteration, and fragmentation (Brook *et al.,* 2003) brought such impact on a broad range of species population declines (Pimm *et al.,* 2001). In the Philippines, threats include unsustainable agriculture and deforestation, pollution of streams and rivers due to industrial settlements, and human harvesting for international export (IUCN Red List, 2018) and diseases, which cause the apparent decline of *Rhinella marina* species in Negros, Philippines according to Alcala (1957).

Anurans, as biological indicators of the health of the environment, are known for their sensitivity to habitat changes and have also been the most threatened species in many terrestrial and freshwater domains (Fabricante and Nuñeza, 2012). Anurans inhabit different ecosystems close to the susceptibility of a danger in which competition, predation and anthropogenic activities may occur and as a suggested bio-indicator of the environment, it is therefore imperative to not just conduct biodiversity research on these species but also focus on its diet analysis as it may provide valuable data in the sustainability of anuran population (Ynot *et al.,* 2017).

Unfortunately, in the Philippines, the dietary information of anurans remains scarce such that only a few published studies have been conducted (Ates *et al.,* 2007; Fabricante and Nuñeza, 2012) and such that the majority of the studies mostly focus on species richness and population count which often does not present data on diet pattern of anuran species and besides there are still unexplored areas in the Philippines that have not been studied especially in Mindanao which is the second largest area in the country.

Thus, the importance of dietary information in understanding the impact of habitat modifications on fluctuating populations should be known (Ates *et al.,* 2007) and is true for the species that inhabit endangered areas. Moreover, the dietary information will be useful in the Philippines as it supports not only a higher level of endemism but also one of the most biodiversity-endangered areas of the world (Conservation International, 2006).

In this study, the researchers aimed to examine the diet and food preferences of the chosen species from the mining area in the municipality of Gango, Libona, Bukidnon, which consists of some open fields where residential areas are present. The data information that will be gathered may add to the available literature studies regarding the diet patterns of *R. marina* and its implications for other organisms where it feeds, given its invasive nature.

Materials and methods

Research setting

The study was conducted in the two selected sampling points located in the mining area in Gango, inside the municipality of Libona, Bukidnon. Libona is a 2nd class municipality in the province of Bukidnon, Philippines (Fig. 1). The municipality of Libona is situated in the northern part of Bukidnon and approximately 92 kilometers from Malaybalay, the capital city of Bukidnon. It covers a total land area of 374.37 km^2 and has a typical tropical forest climate. According to the 2015 census, it has a population density of 43,969 and consists of about 14 barangays.

Entry protocol

Upon entering the area, the researchers requested a permission letter from the Barangay Chairman to have enough information regarding the area and also for the security and safety of everyone before conducting the research. Before the sampling started,

a gratuitous permit from the DENR office was acquired as a mandatory policy in order to allow the collection of frogs *(R. marina)* in Barangay Gango, Libona, Bukidnon, near the small-scale gold mining. Fig. 2 shows the stream below the tailing pond where the first transect line was established. The site is located from 8˚24'14.3" North to 124˚40'27.3" East. The stream is about 2-3 meters wide, flowing into the Bigaan river, Cugman Basin. The water in the site is turbid and rocky with some bed of small gravels.

The tailing pond is about 1-5 meters in depth and the area is approximately 5-20 m from residential areas. There is a wide canopy cover along the riparian zone. Grasses, leaf litter and land-dwelling organisms were found in the area.

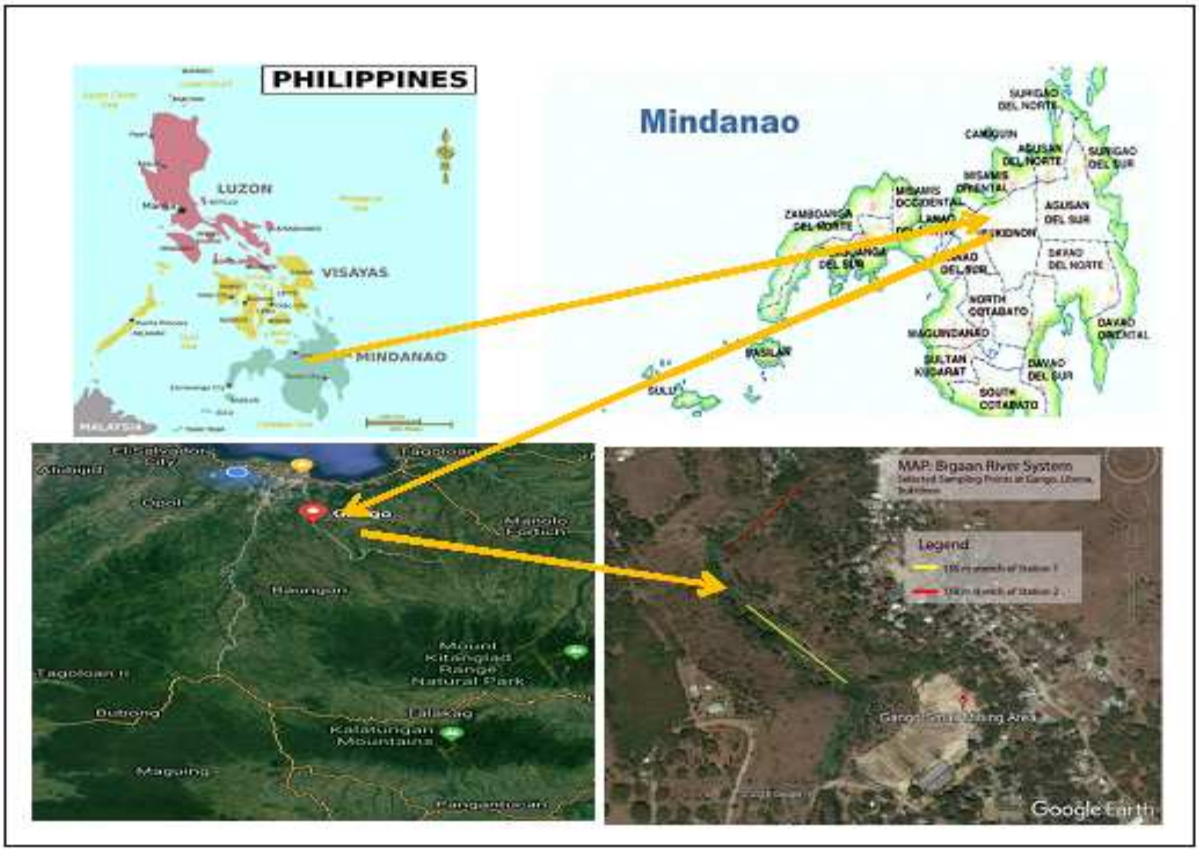

Fig. 1. Maps of a. Philippines, b. Mindanao, c. Gango, Libona, Bukidnon and d. sampling area.

Fig. 3 shows the adjacent second transect in the sampling area labeled Station 2. It is located from 8˚24'16.8" North to 124˚40'31.2" East. The water is also turbid but is mostly rocky compared to the first transect. Canopy cover is denser, but grasses are also visible in the riparian area. The stream ranges from 1-3 meters wide adjacent to the flowing water normally when the rain pours similar to the first area and thus, in this scenario, the diet of a certain organism could be observed.

Toad collection

The researchers collected the *R. marina* species with the locals. The researchers brought headlights and wore protective gear like a face mask, jacket, long pants and boots due to the health risks in the mining area. Captured toads were then carefully placed in a clean, moisture-free bag or in a safe plastic container. Sampling was done in September and December 2018. The toads were collected during nighttime from 6:00-7:00 PM. A 150m transect line was established in each station to sample toads.

Diet and data analysis

After gathering samples, the collected toads were brought to the school laboratory to measure their size using a dial caliper and were slayed using 70% ethanol within (3) three hours. The purpose of

ethanol was to prevent the deterioration of any gut contents in the toad. Next, the toads were rinsed with clean water and placed in a dissecting tray and pinned the legs and forelimbs with their belly (ventral side) facing up. The body wall was slit across from the esophagus to the anus during an examination. After that, the whole gut was removed, separated, and placed in Petri dishes. The contents of the gut were examined under a binocular microscope to determine the diet of *R. marina*. In identifying and determining the diet, identification keys were used. The percentage for each prey was calculated.

Results and discussion

Diet composition of R. marina

The result of the comparison of food items that have been collected in the gut of *R. marina* in each sampling point was summarized in Table 1.

Table 1. Prey item of *R. marina* collected from each station in Gango, Libona, Bukidnon.

Prey Items	Sampling Station 1		Sampling Station 2		Total # of individuals	%
	# of individuals	%	# of individuals	%		
1.Collembola: Springtails	27	23.7	3	9.38	30	20.55
2. Diptera: Flies	1	0.87	-	-	1	0.68
3. Coleoptera: Beetles	8	7.02	2	6.25	10	6.85
4. Diplopoda: Millipedes	17	14.91	9	14.52	26	17.81
5. Oligochaeta: Earthworm	4	3.51	2	6.25	6	4.11
6. Hymenoptera: Ants	30	36.32	16	50.00	46	31.51
7. Isoptera: Termites	27	23.68	-	-	27	18.49
Total	114	100	32	100	146	100

There are seven Orders that were found in the guts of the toad, such as Collembola, Diptera, Coleoptera, Diplopoda, Hymenoptera, Isoptera and Oligochaeta near the mining area. There are 146 individuals of prey items with a higher prey number (114) in Station 1 than in Station 2 (32). In the data presented, the study reveals that there are similarities between the diets of *R. marina* in the two sampling stations near the mining area. Although Diptera and Isoptera are the only taxa that are not present in the second station, this toad still consumes a large amount of prey. Likely, the majority of invertebrates consumed were ants with 31.51% in the two sites. The majority of the food consumed was from the Phylum Hymenoptera (Ants) with 36.32% in Station 1 and 50% in Station 2. It is specifically the ants, which are common in the guts, as reported earlier in the studies of Ahlm (2015) and Hamilton (1948), as a common food source of *R. marina*. Therefore, in this case, *R. marina* was categorized as a generalist in terms of food selection in coherence with the results of earlier studies and the prey collected were mostly terrestrial organisms consistent with the study of Zug *et al.* (1975) and thus, active at ground level during the night.

This toad, *R. marina* consumes anything it can catch such that it eats a variety of animate and inanimate material in terms of its feeding habits (Zug *et al.*, 1975) which takes in much indigestible and inorganic matter with its prey. This consistently supports the idea that such plant, soil, rocks and solid wastes were found within the gut of some individuals of *R. marina* in Sampling Station 1, consistent with the study conducted by Ynot *et al.* in 2017 in the selected areas near human dwellings of Davao, Philippines.

Fig. 2. The study sites in the first transect in the mining area of Gango, Libona, Bukidnon.

On the other hand, soil particulates like small pieces of stones that have been ingested into the gut of the toad are the result of the accidental ingestion of larger prey in which small stones are attached to it and or while ingesting land organisms. Plant matter intake is observable in the gut of *R. marina*. In relation to the ecological dynamics, the need to analyze the diet patterns of *R. marina* in selected sites on and or close to human domains appears to be timely and useful to the existing literature (Ynot *et al.* 2017).

Fig. 3. The study sites in the second transect in the mining area of Gango, Libona, Bukidnon.

Conclusion

Therefore, *R. marina* is a generalist species. It feeds on a wide variety of invertebrates, mostly insects. This study would like to recommend studying the effect of mining on the diet of anurans in the area.

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