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Diet Composition and Determination of Total Mercury in the Gut of *Fejervarya cancrivora* (Gravenhorst, 1829) (Anura Dicroglossidae) in Gango, Libona, Bukidnon, Philippines

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ABSTRACT Mercury toxicity is believed to be a widespread environmental dilemma that has been creating tremendous damages not only to humans, but also to wildlife. Owing to its sensitivity to mercury toxicity, *F. cancrivora* was subjected for the determination of total mercury (THg). The food intake of frogs is claimed to influence mercury concentration on its body, thus its stomach content was also subjected for analysis. The analysis on the gut contents of *F. cancrivora* revealed ten (10) food items in which the insect parts got the highest percentage of occurrence (74.19%). In addition, the total mercury concentration found on the gut of *F. cancrivora* is below the detection limit of <0.02 ug/g which passed USEPA standard of 0.50 ug/g implying that the detected mercury level is still acceptable. This study would like to recommend control and prevention of mercury contamination in the mining area.

KEY WORDS Anuran; disturbed; food item; habitat; threat.

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INTRODUCTION

Globally, mercury (Hg) toxicity is considered as a widespread environmental dilemma. Its primary source comes not only from natural processes (Rasmussen, 1994), but also from anthropogenic activities which includes mining operations (Bonzongo et al., 1996; Wayne et al., 1996; Lacerda, 1997). Mercury (Hg) is known to have no beneficial role in the organisms and no known benefit to biota (Eisler et al., 1987; Eisler et al., 2000). Moreover, it is recognized as one of the hazardous contaminants due to its ability to bioaccumulate and biomagnify and its ubiquity in the environment (Mason et al., 1996; Fitzgerald et al., 1998) which unfortunately creates tremendous threats and poses great adverse effect to humans and wildlife (Wada et al., 2011). Some of the deleterious damages it causes are the developmental abnormalities (Bergeron et al., 2011) and mortality (Unrine et al., 2004). Much of these adverse effects specifically affect the amphibians especially frogs (Unrine et al., 2004). Few studies claimed that there are rising evidences that due to the toxicity of mercury (Hg), the population of amphibians such as frogs are declining or disappearing from its historical ranges (Blaustein et al., 1994; Jennings, 1995; Fisher & Shaffer, 1996).

The tailless amphibians (Anurans) or commonly known as frog is believed to be enormously susceptible to mercury toxicity which resulted to the decline of its population. In addition, it is massively recognized to represent high trophic level and form an important link between the food chains of aquatic and terrestrial ecosystems. Owing to its ecological importance, numerous international studies focused on the assessment of its mercury concentration and contamination

which is believed to pose a threat to their population (Terhivuo et al., 1984; Gerstenberger & Pearson, 2002; Bank et al., 2006; Hothem et al., 2009; Boczulak et al., 2017). Few of these studies specifically investigated the diet or food intake of frog since it does not only contribute to the overall mercury concentration of its body (Hothem et al., 2009; Boczulak et al., 2017), but also it is claimed as the most important route of Hg exposure in amphibians which includes frogs (Unrine et al., 2004). Since the food intake greatly influences the mercury concentration on frog, its dietary pathway known as the gastrointestinal tract or gut (mouth, esophagus, stomach and intestines) which is responsible for its digestion caught the attention of some foreign studies for mercury analysis (Burger & Snodgrass, 1998; Shaapera et al., 2013).

There are numerous published foreign literatures that assessed the mercury toxicity in frogs specifically on edible frogs since some localities utilize it for food consumption. Few of those studies focused on the investigation of mercury contamination on its diet preferences and on its dietary pathway, however studies about it is still scarce in the Philippines. Thus, in order to fill the gap, the study focused on the diet composition and determination of total mercury (THg) on the gut of *Fejervarya cancrivora* (Gravenhorst, 1829) (Anura Dicroglossidae) since its gastrointestinal tract (gut) is the one responsible for digesting its food intake.

The study was conducted in Gango, Libona, Bukidnon, Philippines where a small-scale gold mining area is located and where the adjacent community claimed to utilize the *F. cancrivora* for food consumption.

MATERIAL AND METHODS

Entry Protocol

Letter request was forwarded to the Local Government Unit (LGU) of Brgy. Gango specifically to the Purok Leader and Barangay Captain of the area for the conduct of the study and to ensure the safety of the researchers. After acquisition of the Brgy. Permit, gratuitous permit was acquired from the Department of Environment and Natural Resources (DENR) for the collection of frogs.

Study area

The study was conducted along the riparian zone of Bigaan River which is situated directly below the tailing pond of the small-scale gold mining area in Gango, Libona, Bukidnon, Brgy. Gango is located on the north by Misamis Oriental; on the south by Barangay Kinawe; on the east by Bigaan River and on the western side by Bobonawan River. It is situated at approximately 8°20'N and 124°44'E.

In the study site, two sampling stations were selected based on the microhabitats of *F. cancrivora*, presence of the species and local communities consuming the edible frog. Shallow and deep waters were present where moving and standing water were noticed. Insects like ants and beetles were also seen in the area.

As shown in Fig. 1, the study site was divided into two (2) sampling stations in which Sampling Station 1 (150 m stretch) is directly situated below the tailing pond of the small-scale gold mining

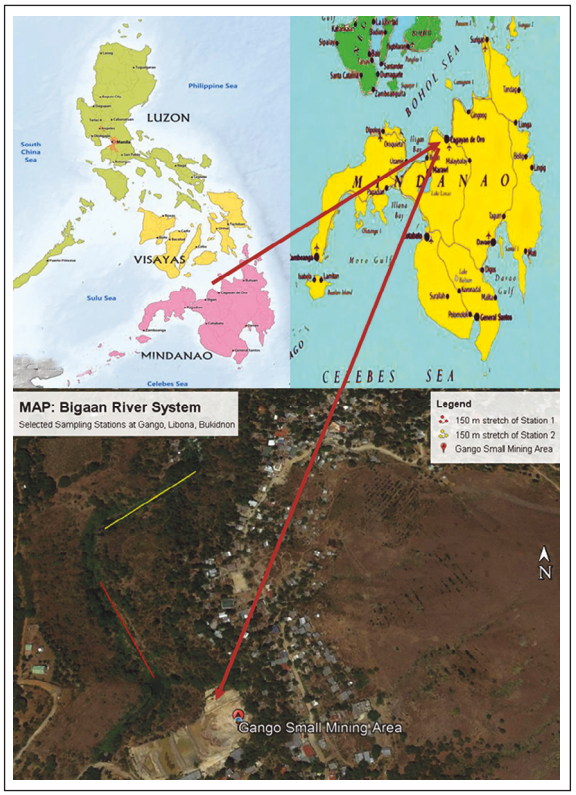

Figure 1. Map of the Philippines, Mindanao and satellite image of the study site in Brgy. Gango, Libona, Bukidnon showing the two (2) sampling stations in the riparian zone of Bigaan River.

area in Brgy. Gango and the Sampling Station 2 (150 m stretch) is approximately 250 m away from the tailing pond.

Samples

The collection of frogs was conducted for four (4) nights within the months of November to December 2018 from 6:00PM to 9:00PM. Together with the local residents, the researchers collected the frogs per sampling station until the number of samples reached 25 per sampling site.

During the night, boots were worn by the researchers for safety purposes due to the presence of mercury in the area. Flashlights and head lamps were used to locate the frogs in their microhabitats along the riparian zone of Bigaan river. Collected adult samples of *F. cancrivora* were initially identified using the descriptive data of Alcala (1982) and by the local residents known to consume the frogs. Sample frogs which belong to adult stage were collected but frogs which are below the required stage were released. In order to minimize contamination, the samples were then double-bagged before it was placed inside the ice bucket. Then, a total of 50 frogs were transported to the Environmental Science Laboratory for the dissection.

Gut Analysis

Before dissection, the samples of *F. cancrivora* species were confirmed by the known experts on frogs in Mindanao. Then, during dissection, the researchers worn hairnet, disposable mask, disposable gloves and laboratory gown to prevent contamination and for safety purposes during the laboratory experiment. Initially, the frogs were euthanized by pithing method in which a rod is inserted through the hole in the head of the frog and thrust caudally through the brain and spinal cord. After that, dissection was done by making an incision in the ventral side of *F. cancrivora* with the use of a sterilized scalpel to expose its digestive tract or gut. The gut was then weighed with the use of analytical balance. Then, the digestive tract was opened carefully to expose its contents. The exposed area was washed with distilled water using a wash bottle to remove the food items and fecal materials which adhered to the walls of the tract. In order to determine the diet, food items which were not immediately identified were viewed under the microscope and photographed. The food items were identified to its nearest taxon.

Determination of Mercury in the Gut

Three replicates were analyzed for mercury contamination from the composite samples (100 g) of gut per sampling point. A total of six (6) replicates of the gut were produced and determined for mercury concentration.

Data Analysis

For the diet composition per station, the percentage of occurrence of each identified taxa was calculated based on the number of individuals per taxa divided by the total individuals of all taxa. Mercury level per station was calculated based on the three replicates per station. The average mercury levels was computed and compared to the standards set by the USEPA.

RESULTS AND DISCUSSION

Diet of Fejervarya cancrivora *in Gango, Libona, Bukidnon*

Diet examination revealed ten (10) food items in the stomach of *F. cancrivora* collected near the tailing pond in the mining area draining towards Bigaan river in Gango, Libona, Bukidnon. *F. cancrivora* was observed to consume a wide range of food items (Fig. 2). Hence, it is considered as a generalist predator and has an opportunistic nature in its feeding behavior (Almeria & Nuñeza, 2013).

It was observed that insect parts comprised the largest portion consumed by *F. cancrivora* and the most frequent food item found in its gut (74.19%). A study by Almeria & Nuñeza (2013) also revealed almost the same food items in the gut of *F. cancrivora* except for Arachnida, Oligochaeta and Scolopendromorpha. Data on this study confirms the study of Elliot & Karunakaran (2009) that the diet of *F. cancrivora* was dominated by insects which are mainly compose of bugs and beetles as the larger items, and a considerable number of small ants and dipterans. The presence of large amount of insect parts in the gut of *F. cancrivora* indicates that the

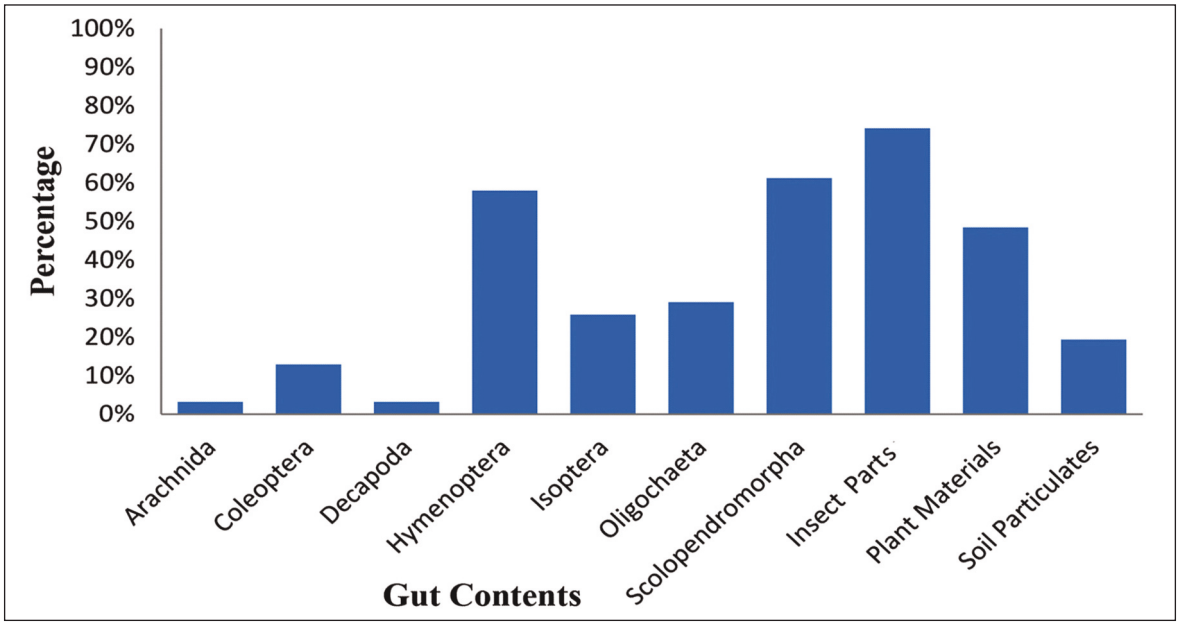

Figure 2. Percentage occurrence of gut contents of *Fejervarya cancrivora* in the riparian zone of Bigaan River.

digestion happens so rapidly that it almost digested all the food items in its stomach.

On the other hand, the least frequent food items on the digestive tract of *F. cancrivora* are the Arachnida (scorpions) and Decapoda (crabs) (3.22%) since the presence of crabs and scorpions were not frequently observed on the sampling site. In freshwater and moist terrestrial environments, amphibians such as frogs are considered as the primary predators of invertebrates, mostly insects. However, they also found to consume plant materials which are present in the digestive tract of *F. cancrivora* such as pieces of tree branches and leaves. The results of this study is similar to Santos et al. (2004), Stebbins & Cohen (1997) and Zug (1993), that *F. cancrivora* species are also found to feed on plant materials and other nonmoving food items.

Mercury Levels on the Gut of Fejervarya cancrivora

Table 1 presents the total mercury (THg) concentration in the composite samples of gut of *F. cancrivora* from two (2) different sampling stations along the riparian zone of Bigaan River in Gango, Libona, Bukidnon. It was found out that the total mercury (THg) concentration in the gastrointestinal tract of *F. cancrivora* from both sampling stations were less than the detection limit of 0.02 ug/g. Therefore, any mercury level below 0.02 ug/g could be as a result of bioaccumulation (Shaapera et al., 2013). Moreover, it was assessed that the mercury concentration of *F. cancrivora* is less than the recommended USEPA standards of 0.50 ug/g for mercury content on frogs (US EPA, 2012) implying that the detected mercury level are still acceptable.

Sampling Station	Replicates-Sample Code	Final Concentration, ug/g	Average Concentration, ug/g	US-EPA permissible limit (0.50 ug/g)
1	R_1-CD1810-3633	<0.02	<0.02	Passed
	R_2-CD1810-3634	<0.02		
	R_3-CD1810-3635	<0.02		
2	R_1-CD1811-3661	<0.01		
	R_2-CD1811-3717	<0.02		
	R_3-CD1811-3718	<0.02		

Table 1. Total Mercury (THg) concentration in the gut of *Fejervarya cancrivora* from two (2) sampling stations long the riparian zone of Bigaan River in Gango, Libona, Bukidnon.

The result shows that even though food intake greatly influences the mercury concentration of the frog, its gut which is responsible for its digestion is not capable of holding the mercury for a long time. The mercury does not stay longer in its stomach

considering that *F. cancrivora* is capable of digesting the food items rapidly due to its short digestive tract, hence the mercury was immediately absorbed and rapidly distributed by the blood, in which only 1% will be deposited and retained in the brain for a long time, and the rest will be transported to the liver and kidneys where it will be excreted through bile and urine (Shaapera et al., 2013).

CONCLUSIONS

Therefore, *F. cancrivora* is a generalist species which feed on a variety of food items mainly insects in the mining area. The food ingested by *F. cancrivora* is a major source of mercury in their guts. Although, the total mercury (THg) concentration on the gut of *F. cancrivora* from both sampling stations were below the detection limit of 0.02 ug/g, indicating that, any mercury level present on the gut of *F. cancrivora* could be a result of bioaccumulation. Finally, the recorded total mercury (THg) concentration on the gut of *F. cancrivora* does not exceed the permissible limit of 0.50 ug/g set by US-EPA implying that the detected mercury level is still acceptable. Based on the results on varied diet composition and minimal levels of total mercury (THg) concentration on the gut of *F. cancrivora* in Gango, Libona, Bukidnon, Philippines, the following recommendations were established. First, to determine mercury levels in the other species of frogs in the mining area in Gango, Libona, Bukidnon. Second, to study the mercury concentration in the food items of *F. cancrivora* like insects. Third, to conduct determination of mercury concentration in the different organs of *F. cancrivora*.

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