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Dietary preference of Alien Invasive Anurans (*Rhinella marina, Hoplobatrachus rugulosus,* and *Kaloula pulchra*) in Butuan City, Northeastern Mindanao, Philippines

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A B S T R A C T

Invasive anuran species (IAS) threaten the native anuran species as they compete for food sources. The diet of the three IAS, *Rhinella marina, Kaloula pulchra,* and *Hoplobatrachus rugulosus*, were collected from different localities in Butuan City. The gut collected from 91 individuals of IAS, i.e., *R. marina* (n=80), *H. rugulosus* (n=10), and *K. pulchra* (n=1) were examined for food items using a dissecting microscope. The *R. marina* had the most recorded prey items, consisting of plant materials, pebbles, insects, and reptile-like species, while *K. pulchra* prefers small and slow-moving insects. *Hoplobatrachus rugulosus,* which also preferred insects on their diet, exhibited frog predation. Among the food categories, plant materials (56%) had the highest percentage, followed by invertebrates (47%), other organic and inorganic materials (12%), and vertebrates (1%). Formicidae, Coleoptera, and Odonata have the highest percentage among the invertebrate prey types. The assessed amphibians feed on locally available prey, generally insects, and suggest potential competition for food resources with native anuran populations.

Keywords: *Invasive anuran species, food items, generalist*

1 Introduction

The presence of invasive anuran species (IAS) competes with native species for essential resources, which could lead to the population decline of native species (Mayer et al. 2015). According to Brown et al. (2013), these introduced species act as competitors for food sources and space. IAS are voracious predators feeding on a wide array of diets, including their juveniles and individuals of other native anurans. Invasive anuran species are widely dominant in the area compared to native species, where their diet mostly overlaps since both invasive and native anurans prefer the same habitat with proximity to water bodies (Mohanty & Measey 2018). The increasing population of invasive anuran species in ecosystems was intentional and accidental, initially used for pest control. Still, countless subsequent introductions to other places in the country have indeed transpired to the invasion of IAS. Human activities such as the commercial production of alien species and pet trades also increase the likelihood of the successful dispersal of this IAS (Diesmos et al. 2006). Urbanized areas in the Philippines, such as Davao, and Butuan City, reported a high relative abundance of invasive species (Gersava et al. 2020; Solania et al. 2020).

Rhinella marina (Linnaeus 1758), an introduced species for pest control (Joshi 2006), is widely distributed from Luzon to Mindanao, thriving in human-altered landscapes (Diesmos et al. 2015).

They are known as generalist feeders (Solania et al. 2019); even a skink was recovered from its stomach (Jabon et al. 2019). This species switches to intensive cannibalism when there is a high dry season and a lack of alternative prey, such as insects (Pizzato and Shine 2008). *Rhinella marina* possesses a large parotid gland that produces toxins and is known to intoxicate its predators, such as the case of pit viper (*Trimeresurus cf. flavomaculatus*) that leads to the death of native species through lethal toxic ingestion (Dela Cruz et al. 2020).

Hoplobatrachus rugulosus (Weigmann 1834), commercially produced for consumption, and *Kaloula pulchra* (Gray 1831), an introduced species for pet trades, are both non-native, ubiquitous, and synanthropic species. They inhabit water bodies near human settlements, disturbed areas commonly encountered during the rainy season (Cruz et al. 2018; Venturina et al. 2020). *Hoplobatrachus rugulosus* is a voracious insectivore, an opportunistic feeder, and potentially harmful to native and endemic wildlife such as colubrid snakes (*Calamaria gervaisii*) (Diesmos et al. 2006). *Kaloula pulchra* mostly consumes crawling and fossorial insects (Gersava et al. 2020).

These three IAS species, *R. marina, H. rugulosus,* and *K. pulchra,* were reported inhabitants of urbanized areas of the Philippines (Gersava et al. 2020; Solania et al. 2020; Salo and Solania 2022). The opportunistic and generalist feeding behavior of these IAS enables them to adapt to the highly disturbed area (Jabon et al. 2019; Solania et al. 2019). However, the dominating presence of these IAS has brought consequences, especially in competition with native species for food items and space. IAS dominates human-altered habitats such as Butuan, a highly urbanized city in Caraga Region, Philippines. Some localities still need more information on invasive anurans species for their diet preference. This study describes the dietary preference of alien invasive anurans in urban and residential areas of Butuan City, Philippines.

2 Materials and Methods

The study area and collection of samples

Before collecting amphibians, a Wildlife Gratuitous Permit (R13-2021-21) was secured from the Department of Environment and Natural Resources Regional Office XIII (DENR-RO13). The samples were obtained from three barangays in Butuan City, Agusan del Norte, Philippines. Butuan City is a coastal, highly urbanized area in the Caraga region. It is the region's commercial, industrial, and administrative center, with a population of 372, 910 as of 2020 and an area size of 81, 662 hectares located in the northeastern part of the Agusan valley, Mindanao (Mapa, 2021). (Figure 1). The mapping of plots in each sampling site was obtained through Arcmap.

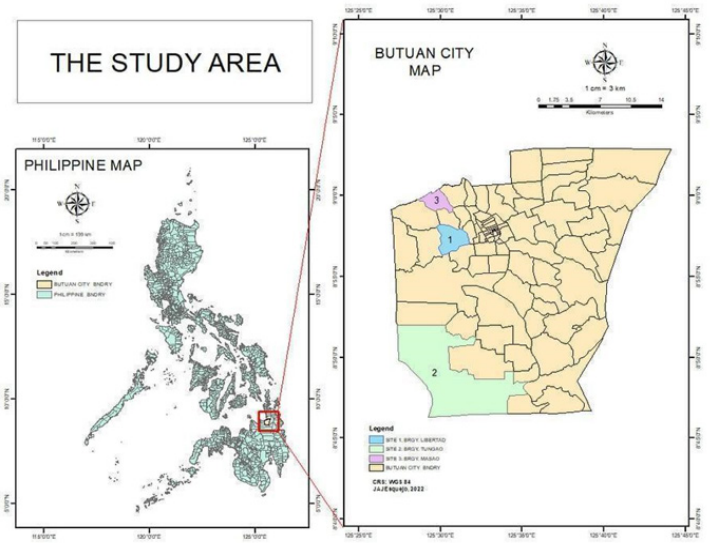

Figure 1. Map showing the sampling sites of selected barangays of Butuan City, Agusan del Norte, Philippines.

Site 1 was located in Brgy. Libertad (8°56'55.0"N and 125°30'44.9"E) an urbanized barangay with many commercial buildings, subdivisions, public markets, schools, hospitals, and a few agricultural areas. Site 2 was situated in Brgy. Tungao (8°46'47.6"N and 125°30'40.0"E), a small rural barangay and an agroforestry site. It is characterized by vegetation that includes falcata (*Paraserianthes falcataria*), rubber tree (*Havea brasiliensis*), coconut (*Cocos nucifera*), rambutan (*Nephelium lappaceum*), and lanzones (*Lancium parasiticum)*. Site 3 area was in Brgy. Masao (9°00'10.2"N and 125°29'23.0"E), is a coastal area with human settlement, beach resorts, and an adjacent river system.

Within each barangay, extensive and opportunistic sampling was conducted to collect invasive anurans from 7:00 to 9:00 P.M., using handling equipment such as gloves and wooden sticks, then placed inside a net bag (DENR-BMB 2017; Jabon et al. 2019). In the agricultural area of Brgy. Libertad (site 1) sampling was conducted on October 29 and November 5, 2021. In site 2, the agroforest of Brgy. Tungao collection of samples was from February 6 to 8, 2022, and in the residential area of Brgy. Masao (site 3), the sampling was conducted from February 18 to 24, 2022. Available taxonomic keys, published articles, and other online sources were utilized for identifying the captured invasive anurans (Solania and Gamalinda 2018; Diesmos et al. 2015).

Gut content analysis

The methods of Jabon et al. (2019) were employed in this study to document the diet composition of collected IAS. Individuals were first pithed to immobilize. Pithing is a technique used to destroy the brain and is relatively painless to the frog (Back et al. 1915). Samples were then eviscerated by opening a longitudinal incision from its snout to the vent, and internal organs were collected. The stomach and intestine were sliced open with sharp blades and a scalpel until the contents were easily collected.

Each prey item was examined under the dissecting microscope to identify the diet of IAS. Food items were classified based on their taxonomic classification (Thyssen 2009; Almeria and Nuñeza 2013) and categorized into four food categories; invertebrate, vertebrate, plant materials, and organic and inorganic materials (Solania et al. 2019). Items such as ants, beetles, cockroaches, and other invertebrate prey were categorized under invertebrates, while vertebrate preys, including its bones and other body parts remnants, were also grouped. Plant fragments, such as leaves, flowers, fruits, and twigs, were considered plant matter. Plastic fragments, pebbles, feathers, and hair strands were categorized as inorganic and organic materials.

The Frequency of Occurrence (FOO) was utilized to identify every rate of identified food items that appeared per stomach of IAS and to evaluate the frequency of food items in every stomach with content analysis. The FOO is calculated as:

$$FOO = \frac{Number\ of\ stomach\ an\ item\ appeared}{Total\ number\ of\ stomach\ with\ content} x\ 100$$

The food items were categorized into “c” as constantly occurring (>50% total stomachs per species); “s” as secondary (25-50% total stomachs per species), and “a” for accidental (<25% total per stomach species) (Jabon et al. 2019).

The Degree of Food Preference (DFP) index was used to deduce the consumption of a particular food item from the counted gut components (Braga 1999 as cited in Jabon et al. 2019). Food items were ranked by categories 1 to 4. The maximum value was four (4) when only one food group was present in the stomach; three (3) as the most abundant if more than one food group was obtained; two (2) to secondary; and one (1) to the occasional occurrence of the food item. Food items were then categorized as highly preferential (3<DFP<4), preferential (2<DFP<3), secondary (1<DFP<2), and occasional (0<DFP<1) (Jabon et al. 2019). The DFP was calculated using the formula:

$$DFP = \frac{S_i}{N}$$

where, S_i is the sum of values given to food items in the guts, and N is the total number of guts of each species analyzed.

3 Results and Discussion

Frequency of Occurrence and Degree of Food Preference

Gut content analysis (Table 1) was performed to identify the frequency of occurrence and degree of food preference in the 91 individuals of the three alien invasive anuran species, namely, *Rhinella marina* (N=80), *Hoplobatrachus rugulosus* (N=10), and *Kaloula pulchra* (N=1) collected in urban and residential areas of Butuan City (Figure 2) from October to November 2021, and February 2022.

Invertebrates were frequently occurring in the examined IAS. *R. marina* consumed all food items, which varies from the commonly occurring Formicidae (FOO=54%), Coleoptera (FOO=30%), Polydesmida (FOO=11%), Blattidae (FOO=10%) to less common food items such as Vespidae (FOO=6%), Gastropoda (FOO=6%), Spirobolida (FOO=2%), and Odonata (FOO=1%). *H. rugulosus* consumed four food items such as Formicidae (FOO=4%), Coleoptera (FOO=2%), unidentified insect fragments (FOO=2%), Odonata (FOO=1%) and Anura (FOO=1%). The *K. pulchra* only consumed food items such as Formicidae

Table 1. Frequency of occurrence (FOO; expressed as %) and Degree of Food Preference (DFP) of gut contents in the collected invasive anuran species from the three selected barangays of Butuan City, Philippines.

Diet category	*Rhinella marina* N = 80		*Hoplobatrachus rugulosus* N = 10		*Kaloula pulchra* N = 1	
	FOO	DFP	FOO	DFP	FOO	DFP
Invertebrates						
Class Diplopoda						
Order Spirobolida	2^{a}	0.03^{o}				
Order Polydesmida	11^{a}	0.1^{o}				
Class Insecta			2^{a}	0.2^{o}		
Order Hymenoptera						
Family Vespidae	6^{a}	0.1^{o}				
Family Formicidae	54^{c}	0.6^{o}	4^{a}	0.4^{o}	1^{a}	1^{s}
Order Diptera						
Family Muscidae	2^{a}	0.02^{o}				
Family Drosophilidae	1^{a}	0.01^{o}				
Order Blattodea						
Family Isoptera	4^{a}	0.05^{o}				
Family Blattidae	10^{a}	0.2^{o}				
Order Coleoptera	30^{s}	0.3^{o}	2^{a}	0.2^{o}		
Order Hemiptera	7^{a}	0.1^{o}			1^{a}	1^{s}
Order Orthoptera						
Family Gryllotalpidae	6^{a}	0.07^{o}				
Family Acrididae	1^{a}	0.01^{o}				
Order Odonata	1^{a}	0.01^{o}	1^{a}	0.1^{o}		
Order Lepidoptera						
Family Noctuidae	9^{a}	0.1^{o}				
Family Crambidae	9^{a}	0.1^{o}				
Class Gastropoda	6^{a}	0.1^{o}				

Note: FOO: Constant (c): >50%; Secondary (s): 25-50%; Accidental (a): <25%; DFP: hp-highly Preferential (3<DFP; p- preferential (2<DFP<3); s-secondary (1<DFP<2); o-occasional (0<DFP<1).

Continuation Table 1. Frequency of occurrence (FOO; expressed as %) and Degree of Food Preference (DFP) of gut contents in the collected invasive anuran species from the three selected barangays of Butuan City, Philippines.

Diet category	*Rhinella marina* N = 80		*Hoplobatrachus rugulosus* N = 10		*Kaloula pulchra* N = 1	
	FOO	DFP	FOO	DFP	FOO	DFP
Vertebrates						
Class Reptilia	1[a]	0.01[o]				
Class Amphibia						
Order Anura			1[a]	0.1[o]		
Plant matters						
Leaves	71[c]	1[s]	5[a]	0.5[o]		
Flowers	3[a]	0.04[o]				
Twigs	8[a]	0.2[o]				
Fruits	26[a]	0.3[o]	7[a]	0.6[o]		
(In)organic materials						
Pebbles	46[s]	0.5[o]			1[a]	1[s]
Plastic	3[a]	0.03[o]				
Feather	12[a]	0.14[o]				
Hair strand	8[a]	0.1[o]				

Note: FOO: Constant (c): >50%; Secondary (s): 25-50%; Accidental (a): <25%; DFP: hp-highly Preferential (3<DFP; p- preferential (2<DFP<3); s-secondary (1<DFP<2); o-occasional (0<DFP<1).

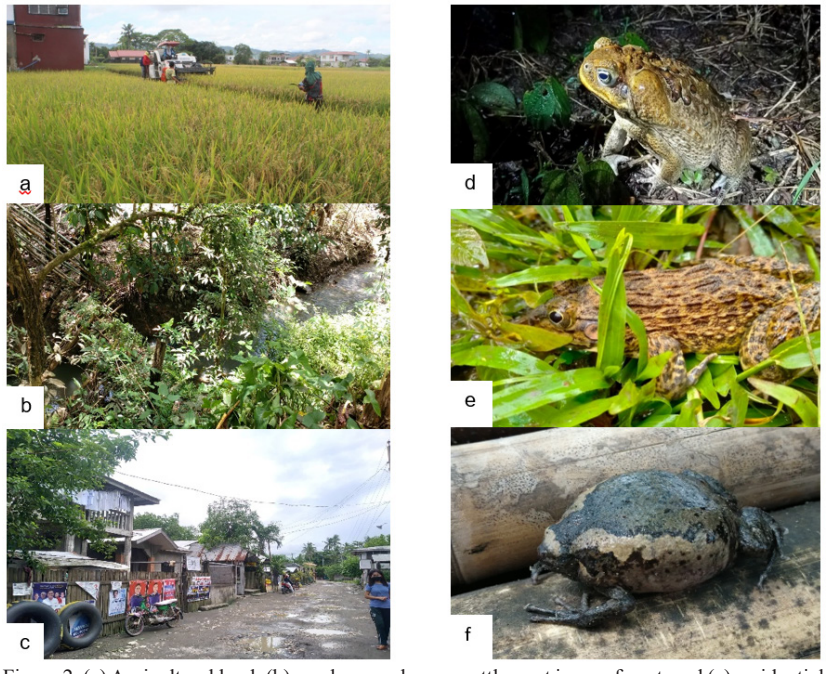

Figure 2. (a) Agricultural land; (b) creek near a human settlement in agroforest, and (c) residential were the habitat types of the three invasive anuran species, namely (d) *Rhinella marina,* (e) *Hoplobatrachus rugulosus*, and (f) *Kaloula pulchra.*

(FOO=1%) and Hemiptera (FOO=1%). Hence, examined IAS are insectivorous, consuming different species of Formicidae, Coleoptera, and Hemiptera.

There were various food items found in the stomach of invasive anuran species, such as ants, beetles, millipedes, mole cricket, plant materials, birds' feathers, pebbles, frogs, and reptile-like species. It indicates that anurans are known for their generalist feeding behavior that consumes a wide range of invertebrate prey (Apayor-Ynot 2017).

The recorded prey items for *R. marina* might be because these species are highly tolerable to all environmental pressures and are the most widespread invasive species. They can adapt to habitats with high anthropogenic activity or highly disturbed habitats. The occurrence of reptile-like species in the diet of *R. marina* was observed. However, it is common to observe this in many anurans (Blanco-Torres et al. 2021). The consumption could be due to the inability of toads to distinguish between reptile-like and other arthropods such as annelids and myriapods. Reptile-like species, such as blind snakes, being fossorial, are occasionally seen on the surface, usually only at night and after rain (Döring et al. 2017). *Rhinella marina* consumes any available food items in the environment.

The relatively short jaws and a shorter feeding cycle of *K. pulcra* (Jabon et al. 2019) could explain why it prefers small and slow-moving prey such as ants.

The diet of *H. rugulosus* primarily consists of small insects and has the most remarkable record of the prey item. A whole frog was found in the stomach of one of the *H. rugulosus* collected from the agroforest habitat of Brgy. Tungao. Nonetheless, the recovered frog from the stomach was no longer identified as it was already partially digested. The occurrence of frogs in the diet is known as anurophagy, frog-eat-frog predation that constitutes cannibalism behavior (Caicedo-Martinez et al. 2021). In Eastern Nepal, frog predation events by *Hoplobatrachus* spp. were observed (Gautam & Bhattarai 2020) because frogs are known as generalist predators consuming a wide range of prey, including other frogs. The species diversity could influence this in habitat, invasiveness of anuran, and body size (Measy et al. 2015 as cited by Gautam & Bhattarai 2020). This event may have significantly influenced the declining population of native anurans.

Percentage of diet categories of invasive anuran species

A high percentage of plant materials (56%), such as fruits, twigs, and flowers, were recorded in all samples, followed by invertebrates or the insect food category (31%). The Formicidae and Coleoptera were the most frequently encountered insect food item. Inorganic and organic food categories (12%), such as pebbles, feathers, hair strands, and plastic fragments, were ranked third in occurrence. The vertebrates were the least food group recorded from the samples, including a frog and reptile-like species. Although the ingestion of plant materials was frequent, this food category is considered incidental because anurans are known to be opportunistic feeders that prey on any smaller organism that is present in their line of vision. Thus, they are considered insectivores and carnivores (Ynot et al. 2017; Browne 2009 and Toft 1995, as cited by Salo and Solania 2022). However, a new study suggests that they are generalist species organisms (Salo and Solania 2022). Another reason is the interaction of several invertebrates with plants, which may also apply to ingesting small stones or pebbles. Consuming plant materials may aid in eliminating parasites by providing an additional water source to avoid desiccation (Gersava et al. 2020).

Invertebrate diet among the three invasive anuran species

The feeding behavior of anurans can be ant specialist and non-ant specialist (Toft 1980). The ant specialists are poisonous and active forager anurans that feed on numerous small prey. In contrast, the non-ant specialists are cryptic, sit-and-wait foragers that feed on a few large preys. The wide prey range, shared habitat, and ideal time of activity (Santos et al. 2004) are some of the reasons why there is a similarity in the diet of invasive anuran species in the present study.

Among the insect food items, Formicidae, Hemiptera, and Gryllotalpidae (Figure 3) were the commonly observed diets of the three IAS. Formicidae, one of the abundant insect families, are social insects that usually navigate in groups known as colonies. They dwell in soils, leaf litter inhabitants, and some are associated with plants (Lopes et al. 2017). This behavior and characteristic of ants and the foraging feeding behavior of IAS may be one reason for having dominant

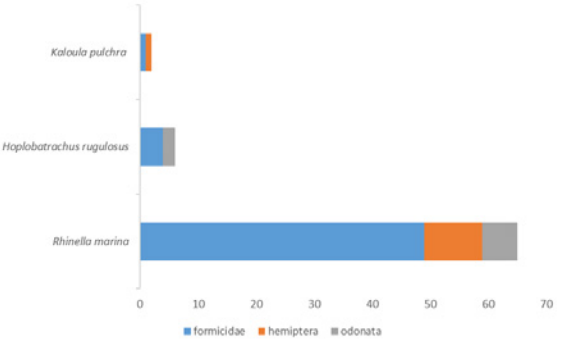

Figure 3. Invertebrate food items among the three invasive anuran species.

invertebrates in their diets. The large arthropod population in the tropical region and the sit-and-wait foraging strategy of invasive anuran species (Gersava et al. 2020) results in the portion of ants, beetles, and mole crickets in the diet of IAS in this study.

4 Conclusion and Recommendations

The diet analysis showed that *Rhinella marina*, *Hoplobatrachus rugulosus,* and *Kaloula pulchra* are insectivore species with a diet that primarily consists of insects. This study also showed that *R. marina* is a generalist feeder consuming the most insect food items, plant materials, inorganic materials, and a reptile-like species. Several unidentifiable insect fragments were recovered from the examined guts of IAS. These results suggest that these invasive anuran species present one of the many underlying threats to native wildlife regarding prey availability. The findings of this study could be used to understand IAS's ecology and dietary preference.

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Statement of Conflict of Interest

The authors declare no conflict of interest associated with the submission and publication of this manuscript.

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