AES BIOFLUX

Advances in Environmental Sciences - International Journal of the Bioflux Society

Diet and endoparasites of *Rana grandocula* (Amphibia, Ranidae) and *Limnonectes magnus* (Amphibia, Dicroglossidae) in Mt. Sambilikan, Diwata Range, Agusan del Sur, Philippines

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Abstract. Anurans are recognized as one of the most threatened fauna on the planet, yet poor knowledge on the basic biology of these species is said to impede proper conservation efforts. The Philippines may have one of the most unique anuran faunas in the world, however studies on the feeding habits and endoparasites of anuran species are still scarce. This study examined the diet and endoparasites of *Rana grandocula* and *Limnonectes magnus* using gut content analysis on available voucher specimens: *L. magnus* (21) and *R. grandocula* (16), collected in the forest of Mt. Sambilikan, Diwata Range, Agusan del Sur, Philippines. Nine food items were identified including plant debris observed in *L. magnus*. Results on diet examination indicated the frogs' high dependence on eight orders of invertebrate fauna dominated by Orthoptera. Niche overlap between the two species was demonstrated as well as new records on being intermediate hosts to the cestode, *Diphyllobothrium latum* and nematodes. *L. magnus* showed higher intensity of infection for cestode while nematode infection was higher for *R. grandocula*. Sex and body size were non-determining factors for infection in both species except for the cestode, *D. latum* which showed a significant association with sex (p=0.049) in *R. grandocula*, with male frogs showing higher infection rates than females despite their significantly smaller size (p=0.013). Diet and endoparasites were found to be interrelated, as well as to the habitats of the two species.

Key Words: anurans, conservation, feeding habits, food items, infection.

Introduction. Anurans, being a highly sensitive taxa, are crucial indicators of environmental health and contamination. The Philippines is said to house a high diversity of anuran species with among the highest rates of endemism in the Indomalayan realm (Siler 2012; Diesmos & Brown 2011). Anurans which include the frogs and toads, are sentinel species for environmental disturbances in specialized habitats, but sadly these biological indicators and important energy conveyors up the food chain, are also the most threatened. One estimate suggests that the current extinction rates may be 211 times the background rate of extinction (Calderon & Stabeli 2011). According to the criteria of the International Union for Conservation of Nature, a higher percentage of amphibians are threatened than birds or mammals, with many amphibian populations and species on the brink of extinction (Blaustein et al 2012). Habitat destruction and alteration, overexploitation, alien species, global warming, increased use of pesticides and emerging infectious diseases are among the leading hypotheses thought to underlie anuran declines (Blaustein et al 2012; Calderon & Stabeli 2011; Collins & Storfer 2003; Stebbins & Cohen 1997). Knowledge on the basic biology of the species including habitat preferences and feeding ecology is crucial in employing effective conservation strategies either in the wild or in captivity (Pough et al 2001). However, poor knowledge on the ecology and distribution of this threatened fauna is said to impede the formulation of informed strategies for their conservation and management (Kovacs et al 2010; Alcala et al 2004; Alcala 1986). Dietary information is crucial for the understanding of anuran life history, population fluctuations, the impact of habitat modification on those populations especially for species that inhabit endangered areas, and as an indicator of the quality of the environment in which the amphibians live (Kovacs et al 2007; Santos et al 2004). On the other hand, endoparasites affect survival and health and may also be sources of pathogens (Johnson & Hoverman 2012; Blaustein et al 2012). While the Philippines may have one of the most unique anuran fauna in the world, studies on the feeding habits and endoparasites of anuran species are still scarce. The only existing record for endoparasite in *Limnonectes magnus* (Stejneger, 1909) is *Parapylostoma crooki* (Van de Vusse 1976) while no records exist for *Rana grandocula* (Taylor, 1920) (National History Museum 2012) (Figure 1). In terms of their diet, although some anurans can be considered specialists, they are usually reported as exhibiting generalist and opportunistic feeding habits (Piatti & Souza 2011). Alcala & Brown (1998) recorded invertebrates as primary components in the diet of both *L. magnus* and *R. grandocula*. Ates et al (2007) in their study on the diets of six anuran species found that Orthoptera is both the constantly frequent occurring and preferential food item in *R. grandocula*. *L. magnus* however, was not included in the study.

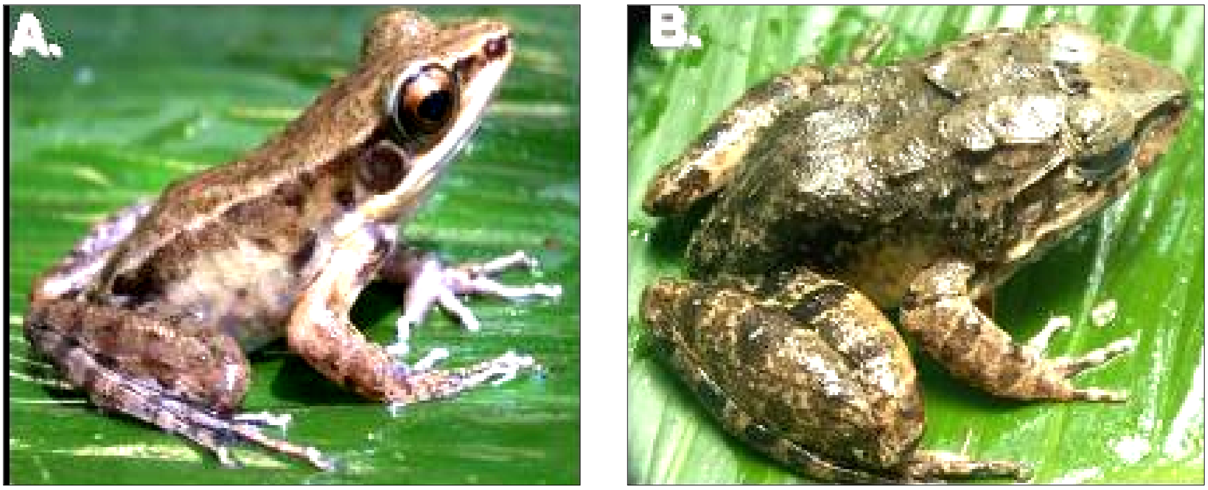


Figure 1. *Rana grandocula* (A) and *Limnonectes magnus* (B).

R. grandocula is an endemic least concern species but loss of the rainforest and the pollution of mountain streams and rivers seem to threaten the population (AmphibiaWeb 2012). *L. magnus* on the other hand is a near threatened species of the family Dicroglossidae found to be distributed in the Philippines as well as on Sulawesi, Indonesia. However, human exploitation and rapid habitat loss had threatened the population to a significant decline (Diesmos et al 2004). Conservation efforts for both species include only a few protected areas covering its range. This study seeks to provide information on the diet and endoparasites of these two species in the hope to supplement data vital for their conservation and management as these factors crucially affect the survival of a species.

Materials and Methods

Anuran sampling. Sampling was done in Mt. Sambilikan, which is a part of the Diwata range in Agusan del Sur, one of the key conservation sites in the country (Figure 2). The land area was classified as an ancestral domain under the administration of three local chieftains. Collection of samples was conducted from October 24 to November 20, 2006 employing the cruising technique (Nuñeza et al 2012).

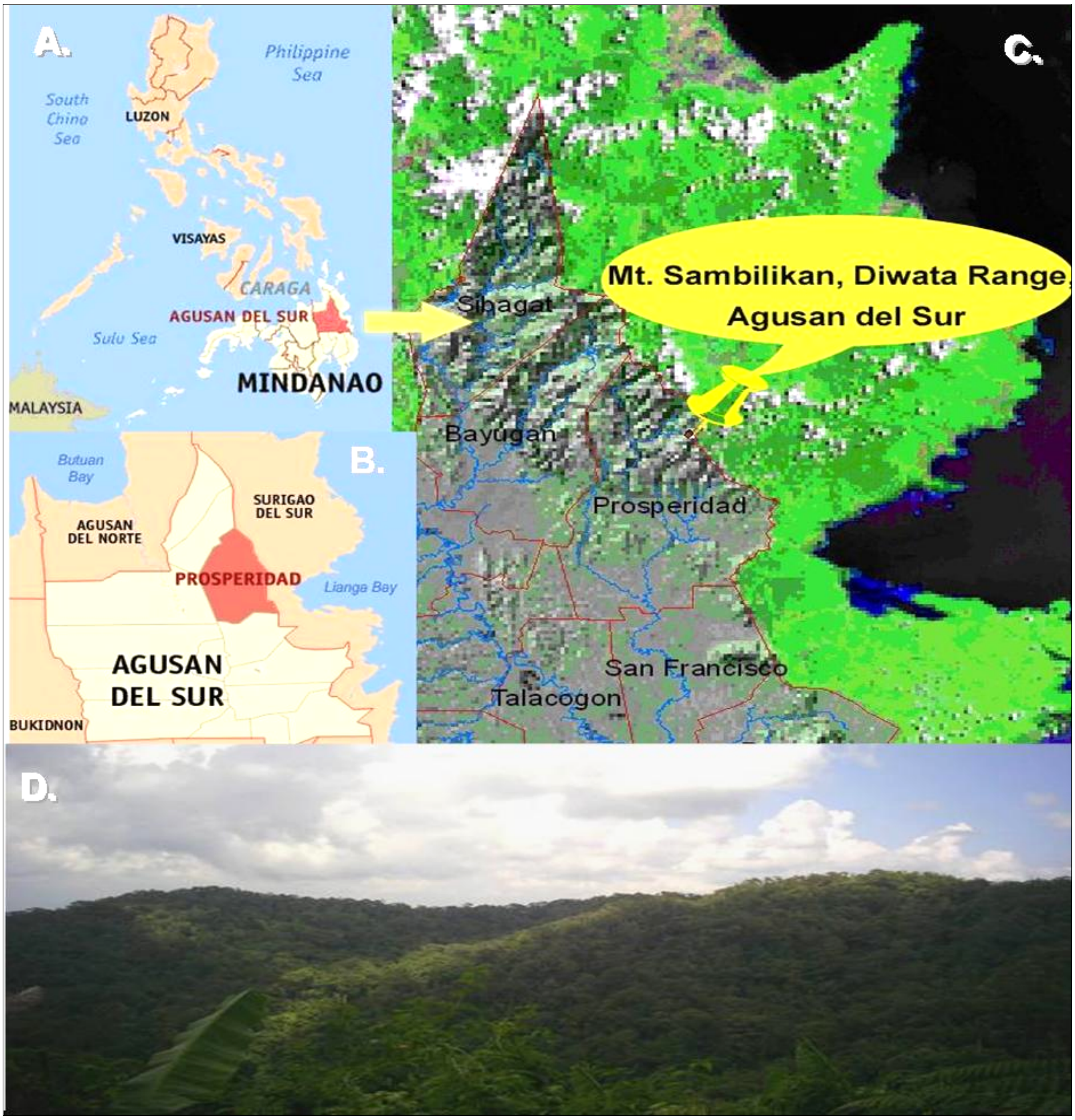


Figure 2. The study area located at Sitio Inagawan, Brgy. La purisima, Prosperidad, Agusan del Sur between 8°34′56″N, 125°53′34″E. (A-B) - Location in the Philippines (www.agusandelsur.gov.ph), (C) - topographical map (www.googleearth.com) and (D) - Panoramic view of Mt. Sambilikan, Diwata range.

Diet and endoparasite examination. Among the 21 frog species found in the area the two most abundant species, *R. grandocula* and *L. magnus* were selected for the diet and endoparasite examination. Available voucher specimens of *L. magnus* (21) and *R. grandocula* (16) were used. 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 cut open. Stored food and fecal material were collected, examined, and identified under a dissecting microscope. Percentage composition and percentage frequency of occurrence of prey items were calculated. Pianka's test (Krebs 1989) was used to determine whether there exists an overlap of niche between the two species. Simultaneously, endoparasite presence was noted as well as the degree of infection. Endoparasites observed were identified up to the phylum or if possible Genus and species levels using the system of classification by Levine (Senler & Yildiz 2000). Mean intensity of infection and percentage occurrence per species were determined. Chi-square tests were done to check whether there exists a correlation between sex and size to the presence or absence of the endoparasites.

Results and Discussion

Diet composition. Diet examination revealed nine food items which include plants and the following invertebrates: Crustacea, Arachnida, Blattodea, Coleoptera, Diptera, Hymenoptera, Odonata, and Orthoptera (Figure 3). Plant debris were observed and described to resemble graminae seeds and undigested dicotyledon leaves. Percentage composition values and percentage frequency of occurrence of prey items in *L. magnus* and *R. grandocula* is shown in Figure 4. Grasshoppers (Orthoptera) comprised the largest portion consumed and the most frequent prey item selected for both species.

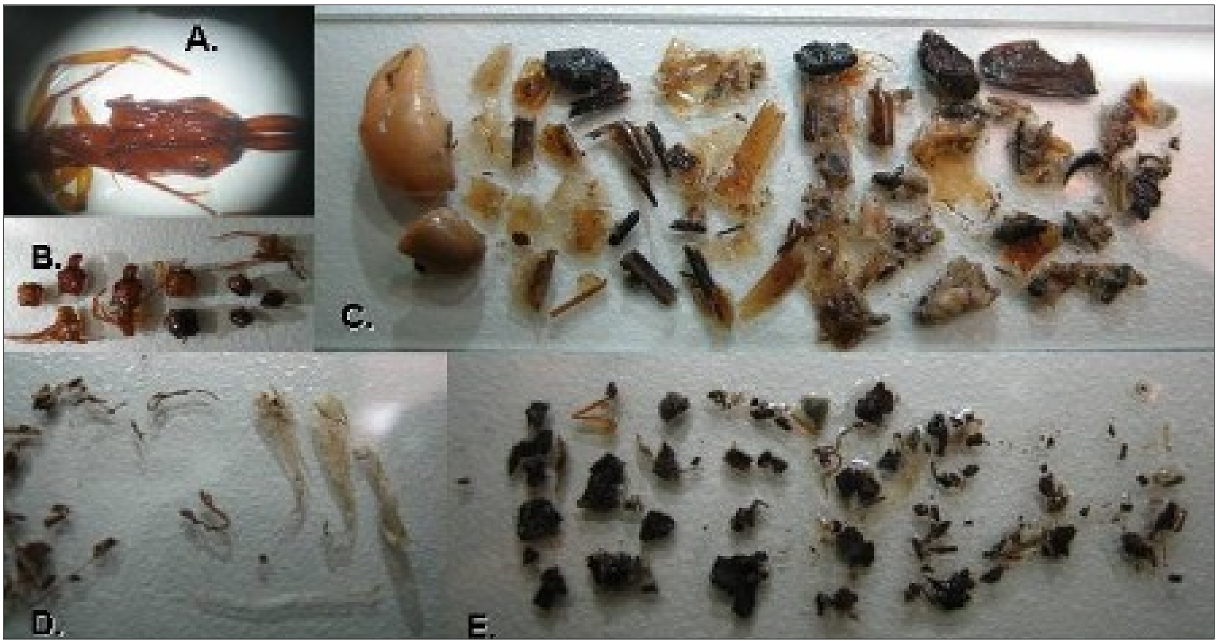
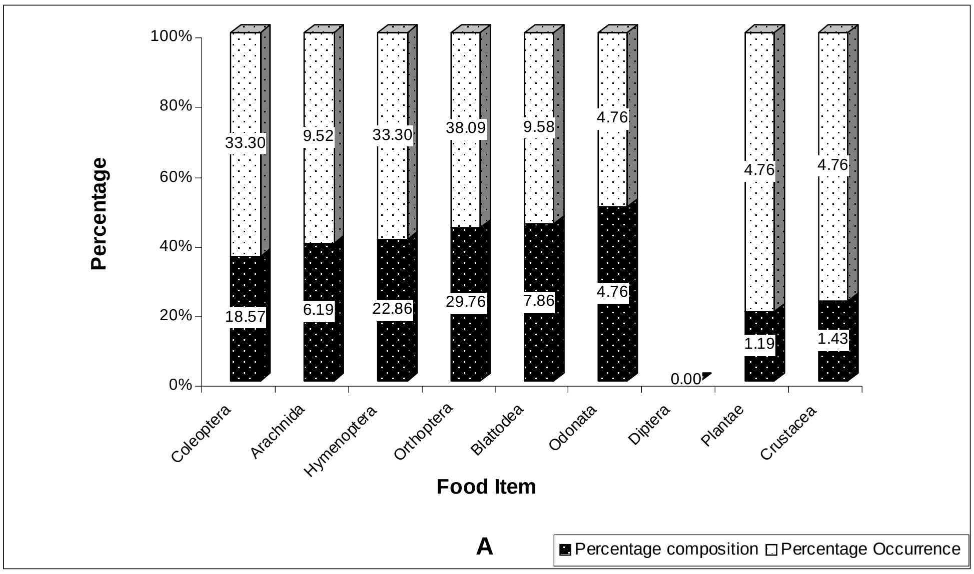


Figure 3. Food items of *L. magnus* and *R. grandocula* showing: (A-B) Hymenoptera, (C) mixed Crustacea and Plantae, and (D-E) mixed insect debris.

Amphibians are recognized as the primary vertebrate predators of invertebrates, majority insects, in many freshwater and moist terrestrial environments, however, some species are also found to occasionally scavenge or rarely feed on plant materials and other nonmoving food items (Santos et al 2004; Stebbins & Cohen 1997; Zug 1993).

Results in this study indicate that *L. magnus* and *R. grandocula* feed mainly on invertebrates as recorded by Alcala & Brown (1998). Data on *R. grandocula* also confirms the study of Ates et al (2007) that Orthoptera dominates as the most frequent and preferred food item for the species. However, since the abundance of each prey item in Mt. Sambilikan was not taken, it is difficult to relate whether grasshoppers (Orthoptera) were greatly consumed because they really were preferred by both *L. magnus* and *R. grandocula* or because they were the most readily available in the area. Abundance and availability of prey is said to greatly influence what is ingested and may tend to conceal preferences (Piatti & Souza 2011; Van Sluys et al 2006; Bull 2003). Although it often may appear that many amphibians have a low level of discrimination in feeding, it must not be assumed that they are wholly lacking in food preferences (Stebbins & Cohen 1997). Furthermore, plant debris were observed as the least frequent food items observed in *L. magnus* maybe due to the frog's difficulty in digesting fiber (Zug 1993) or these plant debris may only have been accidentally eaten together with the capture of prey such as insects. With the increase of the feeding intensity, the accidental ingestion of vegetal fragments also rises (Kovács et al 2007). Santos et al (2004) found that plants were a constant food group for several frog species studied (*Hyla albomarginata, Hyla cf. branneri, Hyla inuta, Phyllomedusa aff. hypochondrialis,* *Leptodactylus natalensis,* and *Physalaemus cuvieri*). Plant contents may help in the elimination of intestinal parasites, provide roughage to assist in grinding up arthropod exoskeletons, or provide nutrients and an additional source of water. Reports on herbivory among tropical frogs is said to be increasing. In the present study, this information on plant consumption may contribute to the understanding of the species' behavioural patterns as vegetation may be used by these frogs not only as a reproductive site, but also as foraging territory. Moreover, variety in prey items may also suggest differences in habitat occupation indicating that data on diet composition can support both ecological and behavioural studies.



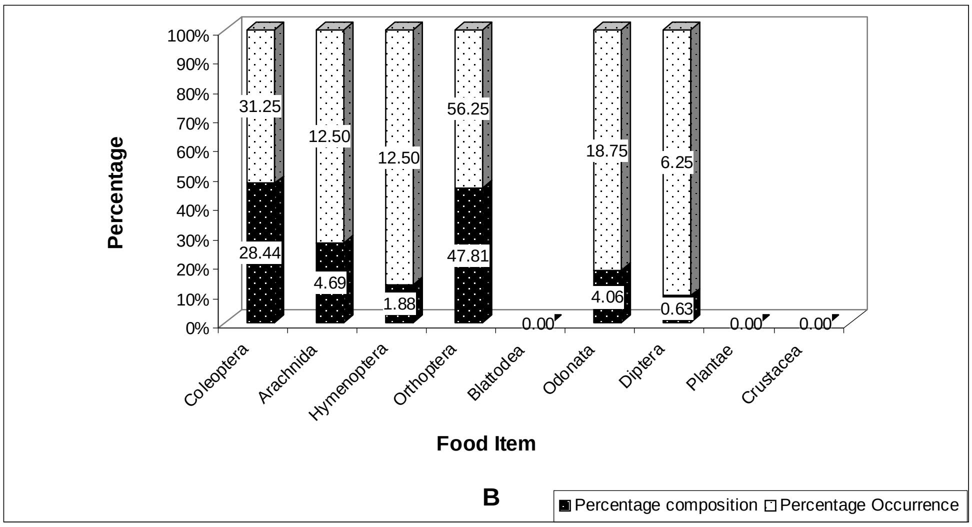


Figure 4. Percentage composition and occurrence of prey items in (A) *L. magnus* and (B) *R. grandocula*.

A computed value of 0.8 (Pianka's test) shows a certain degree of niche overlap between the two species. A high niche overlap may result from enough resource availability in the habitat to satisfy the species or could be a selective force driving species to compete (Piatti & Souza 2011). The more similar species are, the more likely they are to compete as they have more or less the same basic requirements (Drickamer et al 1996). However, many species are also able to coexist even if they are morphologically similar and eat similar kinds of food through differences in feeding behavior (Zug 1993). The similarity between the food consumed by the two species may also be explained through the fact that the two species use the same habitats in the area (Nuneza et al 2012; Alcala & Brown 1998). It is still imperative to consider this possible niche overlap as these species have both important conservation concerns with *L. magnus* being a near threatened species while the *R. grandocula* is endemic. Further studies on the feeding ecology and interactions between anurans within the area are recommended as data could help in future conservation applications. Furthermore, both species were observed to be locally popular as a delicacy, highly prized for their meat. This form of resource utilization may become a major threat if done in large numbers. Hunting of both species must be further investigated to determine whether this needs to be managed sustainably.

Endoparasite examination. Endoparasite examination on the two species showed organisms under phylum Platyhelminthes, subphylum Cestoda and phylum Nematoda (Figure 5). For Cestoda, the organism was classified as *Diphyllobothrium latum*, otherwise known as the broad fish tapeworm identified to be in the plerocercoid stage. The frog being an aquatic organism may be one of the transport hosts of *D. latum*. Frogs eat copepods in the form of small crustaceans. This was confirmed in this study through the diet analysis of *L. magnus* which showed remnants of crabs in its stomach. This may be the entry point of the organism. Being non-definitive hosts, the endoparasite remains as a plerocercoid in its body (Levine 1978). The other endoparasite was identified up to the phylum level only and were classified under phylum Nematoda generally belonging to a group known as the roundworms. Roundworms are highly diverse with up to 10,000 named species living in all sorts of habitats from the soil.

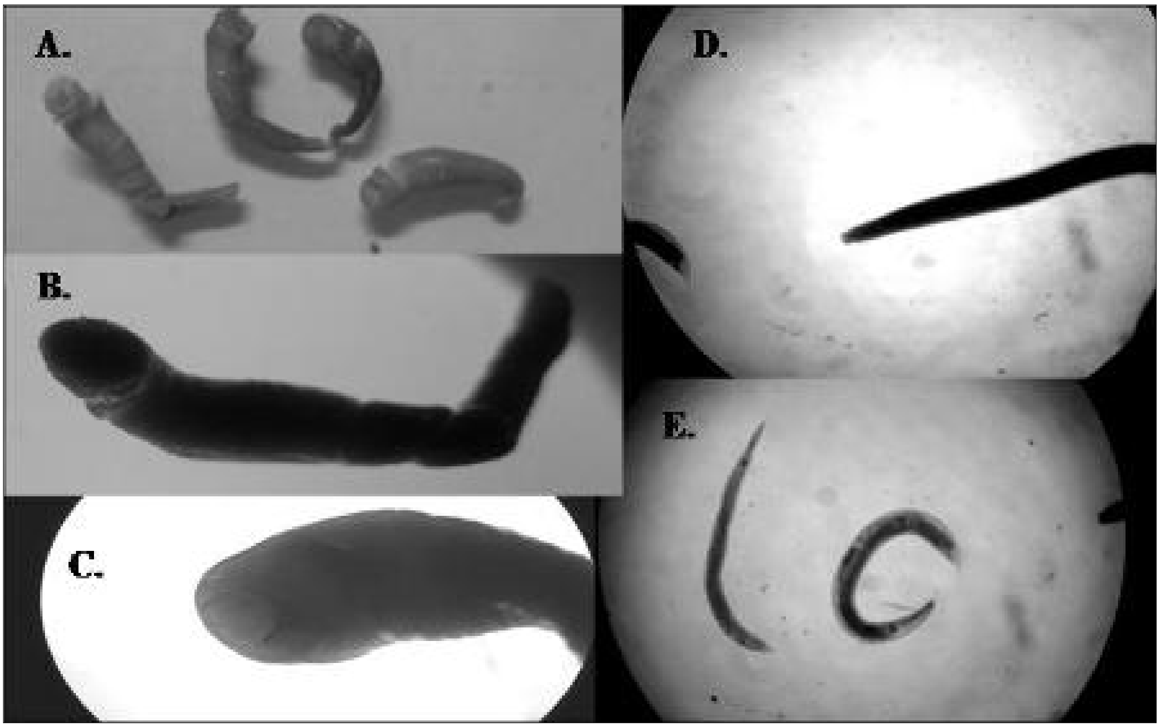


Figure 5. Endoparasites of *L. magnus* and *R. grandocula* showing: Cestoda (A-C) and Nematoda (D–E).

A study by Goldberg et al (2009) examining eleven species of ranid frogs from Papua New Guinea also revealed one species of Cestoda and 18 species of Nematoda among 24 endoparasite species observed. Given their importance as prey or as predators in food webs, herps can be host to a range variety of larval and adult helminth parasite species. Host habitat and dietary preferences play critical roles in the transmission of helminth parasites of herps (Roberts & Janovy 2005).

Of the two species, *magnus* showed higher intensity of infection for cestodes while nematode infection was higher in *R. grandocula* (Table 1). This can be attributed to the interaction between the life cycle of the endoparasites and the ecology of both species. Crustaceans, insects, and mites are recognized to be the most common intermediate hosts of Cestodes (Roberts & Janovy 2005). Examination of the diet of *L. magnus* showed insects and crustaceans as prey items which may be one of the cestode's point of entry. Entry of the endoparasite to its host predominantly is through the ingestion of eggs or larvae into its system while in water (Levine 1978). The preference of *L. magnus* to type IV microhabitats (Gonzales & Dans 1994) which are mainly streams or vicinity of streams, also provides greater opportunity for the cestodes to infect this host. *R. grandocula* in this study was not observed to have crustaceans in its diet but a more comprehensive study could yield conclusive results. *R. grandocula* was observed to inhabit type III microhabitat (Gonzales & Dans 1994) in tree holes, and type IV microhabitat allowing the species to be exposed to a greater variety of possible habitats for nematodes which could also lead to infection. Nematodes greatly abound in a high variety of habitats such as in soil, marine, and freshwater bodies or decomposing matter. In addition, many kinds of insects harbor at least one species of parasitic nematodes and these organisms are also parasitic to the rest of the animal kingdom which does not leave frogs as an exception (Roberts & Janovy 2005).

Table 1

Mean intensity of infection and percentage occurrence of endoparasites in *L. magnus* and *R. grandocula*

Species	*Endoparasites*			
	Cestoda		*Nematoda*	
	Mean intensity of infection	Percentage occurrence (%)	Mean intensity of infection	Percentage occurrence (%)
Limnonectes magnus	3	57.00	1	0.10
Rana grandocula	2	31.25	6	37.50

Sex was not a determining factor for the presence or absence of endoparasites in *L. magnus* with $p = 0.406$ and $p = 0.375$ for the cestode and nematode, respectively. Sex and body size ($p = 0.160$) of the species also showed to be not significantly associated. Sex also did not significantly affect endoparasite infection in *R. grandocula* for the nematodes ($p = 0.91$). However, infection of the cestode (*D. latum*) showed a significant association with sex of these species, showing males to be highly infected than females ($p = 0.049$) although males were found to be significantly smaller in terms of size ($p = 0.013$). According to Van Sluys et al (2006), female frogs have on average more parasites than males and this could be attributed to their much larger size. However, they also argued that the host's sex influence on endoparasite infection is variable among different hosts and parasite species and appears to have more complex causes than simply the body size of hosts. This may be the case for the present study as males were significantly smaller than females for the *R. grandocula* species. Parasites and hosts interact with members of a diverse community that can influence patterns of infection (Orlofske et al 2012). A parasite's distribution is often influenced by many factors such as the host's diet, physiological condition or the presence of other helminthes (Roberts & Janovy 2005). Increase in parasite richness may lead to a decrease in host survival and growth however, these effects of parasite richness on host growth and survival were found to be context dependent emphasizing the importance of community composition and assembly (Johnson & Hoverman 2012). Further analysis should be done to trace the primary factor that may have influenced the greater infection in the males of *R. grandocula* for the cestode species. Body size of *R. grandocula* and infection on the other hand were not significantly associated ($p = 0.465$ and $p = 0.055$ for both the cestode and nematode, respectively).

Conclusion. *R. grandocula* and *L. magnus* showed a generalist diet of nine food items consisting of plants and invertebrates with Orthoptera dominating as both the most frequent and largely consumed prey item. Behavioral pattern of *L. magnus* could be further investigated to see whether vegetation is used not only as a reproductive site, but also as foraging territory. Possible niche overlap between *L. magnus* and *R. grandocula* may be an implication of competition between the two species.

L. magnus and *R. grandocula* are intermediate hosts to the cestode (*D. latum*) and nematodes. The presence of endoparasites was related to the diet and habitats of the two species indicating the interrelatedness of these basic factors for survival.

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Received: 05 August 2012. Accepted: 14 August 2012. Published online: 17 September 2012.
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How to cite this article:
Fabricante K. M. B., Nuñeza O. M., 2012 Diet and endoparasites of *Rana grandocula* (Amphibia, Ranidae) and *Limnonectes magnus* (Amphibia, Dicroglossidae) in Mt. Sambilikan, Diwata Range, Agusan del Sur, Philippines. AES Bioflux 4(3):113-121.