Food preference of the Javan tree frog (*Rhacophorus margaritifer*) in Mount Gede Pangrango National Park and Cibodas Botanical Garden, West Java

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Introduction

The Javan tree frog, *Rhacophorus margaritifer,* is endemic to the island of Java (Fig.1). Its current stronghold includes the mountain range from Mount Gede-Pangrango National Park to Cibodas Botanical Garden in forested habitats at altitudes between 1400 meters above sea level (Ciwalen) to 1800 masl (Swamp svelte) with highest abundance in Cibeureum (1700 masl) (Kusrini et al., 2007; Lubis, 2008). It is also found in Mount Merapi National Park and in East Java around the Ijen plateau. The species is listed as "Least Concern" by the IUCN Red List 2009 (Iskandar et al., 2009) and not listed as a protected species by the Government Regulation 7 of 1999. The population density is believed to be stable (Kusrini et al., 2007) although habitat loss and disturbance are becoming increasing threats to its existence.

Very little is known about the species' behavioural ecology and population biology. Research about the food and feeding behaviour of *R. margaritifer* is important to understand its conservation needs as well as to develop effective conservation interventions. Like many other frog species the Javan tree frog feed on a range of insects, and it is possible that the species is an important predator of several species of insects that are considered pests and potential carriers of diseases. Therefore, protection of the Javan tree frogs' key habitats is critical to ensure the long-term conservation of this endemic species.

This study aims to determine the food and feeding behaviour of *R. margaritifer*, and to estimate the niche width and overlap based on available food resources.

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Methods

Location and Time

Data collection was carried out in the area sampled around the waterfall Cibeureum and Ciwalen (Gede-Pangrango/TNGP Mountain National Park) and the Botanical Gardens Cibodas (KRC) between the months of April-June 2009.

Capturing

73 specimens of *R. margaritifer* were captured between 8:00 p.m. and 22:00 p.m. (10 at KRC, 5 and 58 at the waterfall Ciwalen Cibeureum) using torchlights to identify eye-shine. We measured the snout-vent length (SVL) and body weight, and recorded the type of substrate at capture sites and time of capture. Captured specimens were temporarily stored in plastic bags for further analysis of their stomach contents. After analyzing the stomach contents the frogs were released back on the same site as they were captured.

Figure 1. An adult Javan tree frog, *Rhacophorus margaritifer.* The species is endemic to the Indonesian island of Java. © Luthfia Nuraini Rahman

Stomach flushing
The stomachs of captured specimens were flushed using the method described by Legler and Sullivan (1979). Before stomach flushing frogs were put under mild anesthesia using *Tricaine methanesulfonic* anesthesia (MS-222) with a concentration of 1% (Hirai and Matsui, 2001). Stomach contents were removed by pouring water into the stomach with a small tube following gentle stomach massaging on frogs in a position with the head facing downward. Extracted stomach contents were preserved in 70% alcohol for later analysis.

Food availability
We assumed that *R. margaritifer* feed on primarily insects that are found in the same habitat. Prey specimens were collected by hand and by a light trap using a 52mm diameter candle as light source placed in the middle of the box trap (Borror et al., 1996). Captured specimens were placed in plastic bags and injected with 70% alcohol and preserved in 70% alcohol solution. Preserved insects were taken to the Laboratory of Entomology, Faculty of Forestry for identification and analysis.

Data Analysis
Collected prey specimens were grouped by type (insect: larva and imago, spiders, plants, and other) and identified to order. The abundance of each order, overall composition and prey volume was calculated using the equation for the ellipsoid distribution (Dunham, 1983; Hirai and Matsui, 2000). Correlation between specimens' body size with food volume was tested using Pearson Product Moment correlation equation. Student's t-test was used to test for possible differences in volume of food consumed by male and female *R. margaritifer*.

Results

Food composition
A total of 73 *R. margaritifer* was collected consisting of 65 males and 8 females. The stomach of 33 individuals contained food with the remaining 40 empty. In total, 39 food items were extracted of which 35 items derived from male frogs and 4 females. Seven items were not identified, because the conditions were too poor.
The identified food items belonged to three different Classes (*Arachnida*, *Insecta* and *Gastropoda*) in 11 orders (Tab.1). The result showed that insects (70.27%) constituted the most common prey item followed by *Arachnida* (18.92%) and *Gastropoda* (10.81%). Amongst the insects *Orthopteras* are most commonly consumed (23.08%) (Table 1).

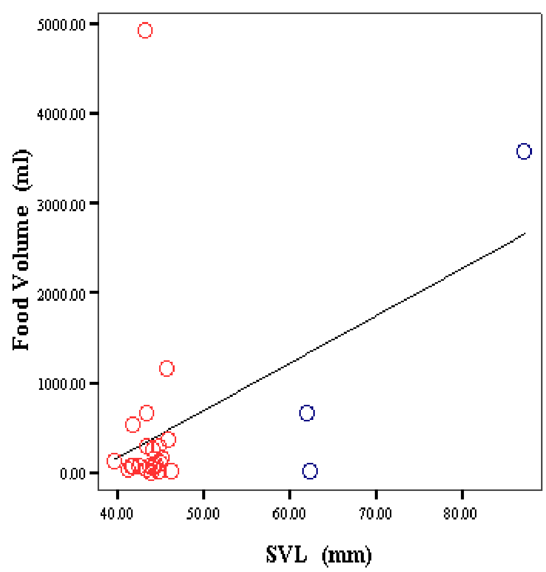

Figure 2. There is a positive correlation between food volume and SVL (Pearson, r = 0.402).

Food volume

The amount of food volume from each individual of *R. margaritifer* ranged from 0.003–6.355ml with an average of 0.937ml. The SVL ranged from 39.65-87.32mm with an average of 46.68mm. Pearson correlation test returned a positive correlation between food volume and SVL (r = 0.402) (Figure 2). The average food volume of females (2.317ml) was higher than the average food volumes extracted from males (0.764ml).

Food availability

The survey resulted in capturing species belonging to nine orders of insects, one *Arachnida* order and one order of *Gastropoda*. *Orthoptera* were the most commonly consumed insect (27,42%) with the *Aranae* and *Pulmonata* reaching only 16,13%. Results of correlation analysis between habitat and abundance of insects in the stomach sample showed a significant correlation (r = 0,678).

The food availability survey from each location revealed that the Cibeureum waterfall area was dominated by *Orthoptera* (26,67%), the Ciwalen area dominated by *Orthoptera* and *Hymenoptera* (50%) and the Botanical Gardens had many *Pulmonata* (60%) (Tab.1).

Food selection

Kendall's correlation analysis suggests that *R. margaritifer* prey opportunistically on available insect species (τ = 0.934). Males (τ = 0.967) have a higher degree of food-variety than females (τ = 0.879).

Niche selection

Applying the Levins index the results indicate that *R. margaritifer* occupies a broad niche in their habitat (BA = 0.642). However, there is a big difference in niche occupancy between male and female *R. margaritifer*. Female *R. margaritifer* tend to utilize narrower niches (BA = 0.167) than male *R. margaritifer* (BA = 0.642).

Tabel 1. The food composition of the Javan flying frog, *Rhacophorus margaritifer*, sampled from Gede-Pangrango National Park and the Botanical Gardens Cibodas between the months of April-June 2009.

Class	Order	*Rhacophorus margaritifer*		Total	Food preference Order (%)	Food preference, Class (%)
		Male	Female			
Arachnida						18,92
	Aranae (adult)	6	-	6	15,38	
	Aranae (eggs)	1	-	1	2,56	
Insecta						70,27
	Orthoptera	8	1	9	23,08	
	Larva	6	-	6	15,38	
	Lepidoptera					
	Hymenoptera	4	-	4	10,26	
	Coleoptera	1	1	2	5,13	
	Blattaria	3	-	3	7.69	
	Embiidina	2	-	2	5,13	
	Isoptera	1	-	1	2,56	
	Dermaptera	1	-	1	2,56	
Gastropoda					10,26	10,81
	Pulmonata	2	2	4		
Unidentified		6	1	7		
Total		**41**	**5**	**46**	**100**	

Discussion

The availability of food constitutes one of the most important features of a specific habitat. It is not only the volume of available food that is important, but also the quality of the food. This is particularly important during the reproductive season. The ability to uptake and process food also depends on the stomach size of an individual, which is usually correlated to the body size. The results of this study indicate that there is a positive correlation between body length (SVL) with a food volume of individual *R. margaritifer* i.e. the larger body size the more food they consume. Since females are usually larger (SVL: 70.54mm) than male *R. margaritifers* (SVL: 43.77) (this study; Hodgkinson and Hero, 2002) it is not surprising that food volumes recorded from female individuals were larger than volumes recorded from males (♀ = 2,317ml; ♂ = 0.764ml). Females also need additional energy uptake during mating season, where they search for the most suitable places to lay eggs and produce a foam-nest (Grzimek, 1974). Apart from needing more energy to sustain a bigger body size, females are more selective in their food choice than males. Although this study did not measure the energy value of selected food items, it is likely that females select prey items with higher energy value than the more opportunistic males.

Many species of amphibians are described as opportunists who take advantage of the resources available in their habitat (Hofrichter, 1999). This study suggests that *R. margaritifer* follow the same pattern, although males and females show varying degrees of feeding opportunism. Elliot tand Karunakaran (1974) reported that *Fejervarya cancrivora* is a very opportunistic species that will feed on *Crustaceae* when insects are scarce. This habit is also recorded in *R. margaritifer* that feed on *Pulmonata* and *Araneae* in addition to insects. *Rhacophoridae* usually forage by ambush where they wait until a suitable prey is within reach of its elastic tongue (Duellman and Trueb, 1994; Grzimek, 1974). Not surprisingly, the most commonly consumed prey items belong to the *Orthopteras* (23.08%) and *Hymenoptera* (10.26%) that usually live on the surface of leaves and are easy to find. Similar preferences are recorded in *L. cruentata* (Kusrini et al., 2007), *Leptobrachium haseltii* (Sasikirono, 2007), *Hyla japonica* (Hirai and Matsui, 2000), *Rana porosa brevipoda* (Hirai and Matsui, 2001), and *Fejervarya cancrivora* (Premo and Atmowidjojo, 1978).

This study suggests that *R. margaritifer* utilizes a diverse range of food resources (Tab.1). This correlates well with the Levin's index that indicates *R. margaritifer* occupies a broad niche, with males (BA = 0.642) occupying a broader niche than females (BA = 0.167). Nurmainis (2000) reported that for the paddy frog, *Fejervarya cancrivora*, individuals with large body size occupied broader niches and were more opportunistic than individuals with smaller body size. Whereas this may also be true for the *R. margaritifers,* this study also reveal that the food preference and niche utilization is also correlated to the specific sex of the individual. When there is a high degree of interactions between organisms and individuals, including competition, predation, parasitism and symbiosis, there is also a significant niche overlap (McNaughton and Wolf, 1990). The *R. margaritifer* in this study exhibited a high degree of niche overlap between males and females. This may explain, along with females' higher need for quality food, the smaller males' tendency to rely on opportunistic foraging, because competing with females for quality food is too high.

The opportunistic feeding behaviour of the Javan tree frog may be key to its long-term survival. While it remains abundant in several key areas, it is dependent on pristine streams for breeding purposes, and its habitat has been steadily declining during the past two decades. More studies about the ecology of this species is important for developing effective long-term conservation interventions.

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