Comparative aspects of the diet of anuran amphibians at different elevations on Gunung Jagoi, Sarawak, Borneo

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A project is submitted in partial fulfilment of the requirements for the Bachelor of Science with Honours
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LIST OF ABBREVIATION

cm : Centimetre

m : Meter

Kg : Kilogram

Km^2 : Kilometre squared

m^2 : Meter squared

SVL : Snout-vent length

W : Weight

Mm : millimetre

Mm^3 : cubic metre

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ABSTRACT

Feeding habit and food preference of the anuran amphibian at different elevation of Gunung Jagoi were studied from 8 to 11 November 2011 and 2 to 5 February 2012. The sampling was conducted of the 100 m streams transect between the elevation of 60 m (Kg Serasot) and 240 m (Kg Bung Jagoi) above sea level. A total of 15 species were captured in Kg Serasot and 10 species in Kg Bung Jagoi. There was a significant difference between the anuran diversity in Kg Serasot and Kg Bung Jagoi ($p<0.05$, Zar t-test). The regurgitated stomach content of 25 species were analysed and it shows that arthropods comprise most diet. The relationship between the diet of frogs with their potential prey available in the environment were done and it is determined that *Megophrys nasuta, Limnonectes kuhlii,* and *Limnonectes malesianus* were assumed to be generalist feeder. Frogs like *Limnonectes leporinus, Leptolalax gracilis, Leptobrachella mjobergi, Hylarana raniceps, Hylarana picturata* and *Microhyla perpava* were classified to be a specialist feeder.

Keyword: Diet, elevation, Gunung Jagoi, Sarawak, Anuran Amphibians

ABSTRAK

Kajian mengenai diet dan pemilihan makanan spesis anura telah dijalankan di ketinggian yang berbeza di Gunung Jagoi pada 8 sehingga 11 November 2011 dan pada 2 sehingga 5 Februari 2011. Pensampelan telah dilakukan sepanjang 100 m anak sungai di antara ketinggian 60 m (Kg Serasot) dan 240 m (Kg Bung Jagoi) di atas paras air laut. Ada perbezaan yang ketara diantara kepelbagaian spesis anura di Kg Serasot dan Kg Bung Jagoi (p<0.05,Zar t-test). Sebanyak 15 spesis telah didapati di Kg Serasot dan 10 spesis di Kg Bung Jagoi. Makanan yang dihadam oleh 25 spesis ini telah dianalisa dan arthropod merupakan sebahagian besar makanan yang dimakan. Diet katak ini berhubung rapat dengan jumlah ramalan mangsa di dalam kawasan sekitar dan Megophrys nasuta, Limnonectes kuhlii,*dan and* Limnonectes malesianus *merupakan katak yang mempunyai diet yang umum. Katak sepperti* Limnonectes leporinus, Leptolalax gracilis, Leptobrachella mjobergi, Hylarana raniceps, Hylarana picturata *and* Microhyla perpava *telah dikenal pasti mempunyai diat yang khusus.*

Kata kunci: Diet, ketinggian, Gunung Jagoi, Sarawak, katak

1.0 Introduction

Amphibians spend part of their life in water and subsequently changing to terrestrial adult, or as an animal that alternate life in and out of water (Duellman and Trueb, 1985). Their distinctive features include: lack of a tail, a short, often stocky body, long hind legs and short front ones, large bulging eyes, and a relatively wide mouth (Inger & Stuebing, 2005). In Borneo, most of the frog species live in the hilly terrain of the lowland forests, which is warm, and humid (Inger and Stuebing, 2005).

There have been a number of studies of the anuran amphibians throughout the years, which mostly on the faunas of lowlands and hilly dipterocarp forests (Inger and Voris, 1993). According to Haas and Das (2011), there are currently 172 species of frogs on Borneo. The families represented include Bombinatoridae (Firebelly Toads), Bufonidae (True Toads), Ceratobatrachidae, Dicroglossidae (True Frogs I), Megophryidae (Litter Frogs), Microhylidae (Narrow-mouthed Frogs), Ranidae (True Frogs), and Rhacophoridae (Afro-Asian Tree Frogs). Each of the Bornean families is divided into group of related species or genera.

Amphibians are known as opportunistic feeders. However, less is known about their feeding behaviour in the wild, except that their dietary mainly comprises insects and arthropods. Some are with specialized diet according to their positions in the mountain. The prey eaten by the frogs at lower elevation may be different with the prey eaten by the frogs living in higher elevations of the same mountain, as insect population might be different species at different elevations. This was shown in a study by Longino and Colwell (2011) which conclude the density of ants started declining rapidly with increasing elevation. Competition between species in acquiring food have presumably resulted in niche overlap, as the motile search predators will likely wander in search of prey. This will eventually lead to niche partitioning.

Frogs are a valuable asset to the environment. Frogs and tadpoles are an important link in the food chain of many ecosystems and do a great job helping control insect pest populations. Burton and Likens (1975) also had stated that amphibians are among the indispensable elements of the ecosystem as they are bridges for energy flow between invertebrates and higher trophic level. They also serve as biological indicators of the health of both aquatic and terrestrial environments as they are sensitive to change.

1.1 Problem Statement

Toft (1980, 1981), and Pough and Taigen (1990), suggested that differences in adult frog diets have resulted from differences in foraging activity or the degree of abundance of prey in the environment. In frogs with generalized diets, prey size usually is positively correlated with body size and head width and large frogs usually eat wider range of prey sizes than small frog. There is little data in other aspect investigate the feeding ecology of the frog. Hence, the following questions need to be answered:

1. Do the prey items at the base and top of Mount Jagoi differ?
2. Do frogs select particular prey or do they eat whatever they encounter? Or would this vary from species to species?
3. Would two individuals of the same species living at the same site eat different prey?
4. Does gape size influence prey selection?

1.2 Objectives

1. To determine food eaten by frogs by flushing their stomachs or dissection of their stomachs.
2. To determine whether frogs eat arthropods as they are encountered in the wild, or whether they select certain types of prey.
3. To determine if there are differences in the diet of frogs found at lower and higher elevations of the same mountain.

2.0 Literature Review

2.1 Amphibian dietary features

Anuran diet is quite different to that shown by other vertebrates. Frog diet always has obvious relations with behavioural, physiological and morphological features, both predators and prey (Schoener, 1986; Huey and Pianka, 1981; Toft, 1981). For example, frogs with generalized diet always have prey sizes positively correlated with their body size and head width, which means large frogs, can eat a wider range of prey sizes than small frog. It is assumed that larger frogs can open their mouth wider than small frogs and therefore can ingest larger prey, but they can eat small prey as well (Emerson *et* al., 1994). In contrast, Wells (2007) assumed that frogs that feed on small, slow moving prey such as ants tend to have narrow heads which consequently have small gape and jaws that are shorter than the head. So, it can be stated that the ability to gape in relation to the size of frog is a limiting factor in the selection of prey (Toft, 1980).

2.2 Foraging pattern

The types of items frogs prey on are often associated with a specific foraging mode. There are two types of foragers, which are sit and wait and active foragers (Toft, 1981). According to Zug (1993), sit and wait predators wait for prey to pass within a capture zone and strike or move to capture the prey while the motile search predators hunts for prey and discover it by searching likely areas. Toft (1981) stated that within each mode, certain trade-offs are apparent. Sit and wait will have low search cost, and almost certainly the physiological cost of digesting the prey is less, at the same time, the larger prey are harder to handle. While, active foragers had much smaller cost of capture per prey item but they have to catch more prey which probably cost more time to digest.

Buytendijk (1941) distinguish between frogs as sit and wait animals and toads as hunting animals. Another example is provided by Feder and Burggren (1992), during the day adult toad (*Bufo bufo*) mostly hide and sleep or sit and wait for prey. Only in warm rainy (moist) weather do they hunt during daytime. These entire examples indicate the complexity of feeding in nature where individual variation and variation in environmental condition may determine whether an amphibian hunts or sit and waits.

2.3 Diet of frogs

Nearly all frogs are either insectivorous or carnivorous as adult (Wells, 2007). Most frogs are generalist feeders consuiming a variety of insects, other invertebrates, or small vertebrates, depending mainly on body size and microhabitat used (Nishikawa, 2000). On the other hand, some frogs specialize in particular types of prey. According to Mitchell and Altig (1983), small anuran amphibians and those with narrow heads such as microhylids often eat particular prey such as mites, collembolans, ants and termite which has small sizes. However, large body frogs often feed on prey of appropriate size and sometimes eat on small vertebrate rather than specializing on particular types of diets (Duellman and Lizana, 1994).

ÇiÇek (2011) investigated food composition of Uludağ frog, *Rana macrocnemis* Boulenger in Bursa Turkey. The diet was determined by extracting the stomach content by forced regurgitation with forceps. This technique was first described by Hirai and Matsui (2001). During the analysis by ÇiÇek (2011), the prey that been eaten by the frog are in the classes Arachnida (Araneae), Chilopododa (Lithobiomorpha), Diploloda (Julida), Insecta (Odonata, Plecoptera, Heteroptera, Homoptera, Hymenoptera, Coleoptera, Diptera, Tricoptera, Lepidoptera and Collembola), and Amphibia (Anura). This result directly show that insect constitute a large proportions on frog diet which indicate that frogs are generalize feeder.

Mahan and Johnson (2007) compared the diets of the *Hyla versicolor* (Gray Treefrog) in breeding habitat and terrestrial habitat in terms of prey abundance and composition. Stomach flushing technique first done by Patto (1998), was modified to facilitate dietary study. It was found that hymenopterans fill most of the stomach content followed by coleopteran, and there a slight difference in number of beetle eaten by frogs in terrestrial habitat and ponds. This is one example that shows that of frogs which have specialized diets.

Maneyro *et* al., (2004) studied diet of the South American frog *Leptodactylus ocellatus* in Uruguay. The frogs were obtained with pitfall traps and supplementary specimens were hand-collected. Using the technique by Schoener (1989) to determine the diet of the frogs where the specimens were dissected and the stomach and the first portion of the intestine was removed. This study used Pianka's index of relative importance to determine the importance each of the prey consumed by the frog and the index show that diet is dominated by coleopteran, followed by orthopteran, spider, hemipterans, ants, isopods, insect larvae, and acari. This study indicates the frog to be a generalist feeder and it is assumed that it has sit and wait foraging mode.

2.4 Resources partitioning

Fu *et* al., (2006) studied the diversity of frog at Henduan Mountain, China. According to Fu *et al.* (2006), total number of frogs peaked at mid elevation. Thus, it can be expect that there will be some competition in acquiring food. Resource partitioning is thought to be the basis of theory for closely-related species, whereby the niche (such as diet, microhabitat and time) of each species differ from all the niches used by other species, reducing competition for food, space and time, and provide evidence of competition (Campbell, 1997). Schoener (1974) deduced that the major purpose of resource-partitioning studies is to analyze the limits inter specific competition place on the number of species that can stably coexist. Cody (1966) stated that in each community, the species separate according to various mixtures of differences in vertical and horizontal habitat, and food type to reduce competition.

Brown (1984) argued that species with broad ecological niches should be a generalist feeder as there will be many prey encountered while foraging while narrow niches restrict a species to a specialist feeder as they number of prey available will be limited. But this statement has been argued by Pianka (1986), in low-productive environment, such as mountain tops, the low abundance of prey item encourage the generalization of diet to maximize returns per unit effort. This may be the mechanism that forces these geographically restricted species to be dietary generalist in order to survive (William *et al.,* 2006). William *et* al., (2006) stated that species with the most geographical overlap with other species was the one with the most distinctive diet. There is a case where the diets of Australian rainforest microhylids have been overlapping but the species still occur in such high abundance due to dietary partitioning (Toft, 1980).

2.5 Technique in analyzing diet

There are many techniques for investigating the diets of frog such as by observing the collected faeces or directly observed the food that been eaten by frogs or by flushing their stomach or through dissecting their stomach (Hirschfeld and Rödel, 2011). But among the four methods, by observing faeces and by directly observed the frog methods are quite irrelevant because the methods are difficult and the prey items might be completely digested or unrecognizable. According to Rice and Taylor (1993), flushing technique are especially useful for dietary analyses of protected species or populations under long term study as this method does not require killing the animals to obtain dietary data.

3.0 Materials and Methods

3.1 Study site

The study was conducted at two elevations of Gunung Jagoi, Bau Sarawak, between 8 to 11 November 2011 and 2 to 5 February 2012. The GPS reading of Gunung Jagoi is N 1°22'0.00" and E 110° 2'0.00. The first sampling conducted at three sites in the Kg. Serasot, Site A is done on the stream that branch into two areas, which are agricultural area and forest area. It has GPS reading of 1°22'27 N and 100.10°2'14 E and elevation of 61 m. Site B is done on the forest area which is higher than site A. At Site C, sampling was done on stream close to site B.

Between 2 to 5th February 2012, a second sampling session was conducted in Kg. Bung Jagoi, at the upper part of Gunung Jagoi with the GPS reading of 1°21'368 N,110°02'213 E. It is done on the stream near the village area at the elevation of 240 m.

Figure 1: Study site at Gunung Jagoi (Google Earth, 2010)

3.2 Field technique

Sampling were conducted at from 1900 hours and finished when transect have reach at the 100 m point. Stream transect was used in this study. Frogs were located and captured by hand. Upon capture, general information such as date, place and time of capture were taken. Insect surveys were done along transects to estimate prey available that may be eaten by frogs. Any insect encountered during the survey were collected and identified. Insecticide was used to immobilize the insect which were euthanized in a killing jar containing chloroform. They were then transferred to small bottles according to the place it were caught.

3.2.1 Handling and identification

Identification of frogs was based on Inger and Stuebing (2005). Amphibians were weighed and their snout-vent length (SVL) and head width (HW) were taken. This is to investigate the relationship between the snout vent length of anuran and the length of prey that it may consumed. The frog was weighed using a Pesola of 10 grammes and 50 grammes, according to their size. A digital calliper is used to measure the SVL of the frog and total length of prey items to the nearest 0.1 mm. The prey items were then transferred in the cans of 10% formalin for further identification. Frog specimens were labelled with the name of species, name of collector, sex, weight, SVL (mm), time of capture and date, elevation of capture, and tagged on their right hind limbs.

3.2.2 Stomach Flushing Method

Upon capture, all the large size frogs are flushed through forcible injection of water through an inserted tube as done by Mahan and Johnson (2007). A 5 mm plastic tube was used to push under the rostrum to force the mouth open and the tube was inserted until it reaches the end of the stomach. Subsequently, 20 ml syringe was used to squeeze the water into the tube to flush the stomach contents. The flushed stomach content was then poured into a petri-dish. The procedure was repeated until no further content were flushed out, followed by one additional flush to ensure the entire contents are removed. The stomach contents were extracted from the water using forceps and the contents was preserved in the bottle of 70% ethanol before being analyzed in the laboratory.

3.2.3 Stomach dissection

To check the efficacy of stomach flushing, most of the frogs flushed were also dissected to examine any remnant food items in their stomachs. The total of prey item found in both dissects and flushing method was gathered in one container for each individual of frog. However, small frogs were dissected directly as the mouths were too small for the tube to be insert. Before it is being dissected, it is euthanized by injecting pure alcohol into the heart of the frog. The stomach of the dissected frogs were cut out and stored in 70% ethanol.

3.3 Food sample analysis

All prey item in the stomach content and those obtained from the flushing were identified to ordinal level or family level whenever possible and numbers of individuals for each prey items were counted under a stereomicroscope, using numerical method of Hyslop (1980). The prey item is counted as individual based on the number of the head observed in the prey samples. Each particular part such as elytra, body segment such as legs and wings and thorax and abdomens were observed using a Motic Image Plus Version 2–0 from Motic China Group Co. Ltd compound microscope with resolution lens of 1600 x 1200 pixels. Identification of foods type was based on Borror *et al.* (1989).

After the prey items individuals had been categorized into order, fluid displacement method was used to investigate the relationship between the volumes of prey items with anuran diet. Volume of prey items were determined by immersing each prey items into 8 ml water in 10ml Pyrex® Vista™ graduated cylinder. The higher volumes of the prey items immersed indicate type of prey preferred by frog.

3.4 Statistical analysis

Species diversity usually refers to the quantity of the species and individuals in a particular habitat (Krebs, 1989). Shanon index was used to determine the species diversity in both Kg Serasot and Kg Duyoh. Modified *DIVERS* Software (Laman, 2001) was used to calculate the Shannon values.

Shanon index's formula:

$$H = - \sum P_i \, ln \, P_i$$

Where:

H =Value of the diversity index

$P_i = N_i / N_i$

ln = natural log

N_i = number of individuals per species

N = total number of individuals of all species

While species evenness is measured to quantify how equal the community between Kg Serasot and Kg Bung Jagoi. The evenness of a community can be represented by Pielou's evenness index:

$$J' = \frac{H'}{H'_{max}}$$

H'= Number derived from the Shannon diversity index

H' *max*= Maximum value of H', equal to:

$$H_{\max} = -\sum_{i=1}^{S} \frac{1}{S} \ln \frac{1}{S} = \ln S.$$

S = Total number of species.

J' value indicates the variance of species between the communities. The less variation in communities between the species, the higher the J' value

Zar t-test (Zar, 1999) was used to test for significant difference in Shannon Weiner index (H') of diversity of anuran between Kg Serasot and Kg Bung Jagoi in Jagoi.

Formula of t-test:

$$t = \frac{H'_1 - H'_2}{s_{H'_1 - H'_2}}$$

Where

$$s_{H'_1 - H'_2} = \sqrt{s^2_{H'_1} + s^2_{H'_2}}$$

The variance of H'_1 and H'_2 is approximated by:

$$s^2_{H'} = \frac{\sum f_i \log^2 f_i - \left(\sum f_i \log f_i\right)^2 / n}{n^2}$$

Degree of freedom for the Zar t-test is denoted as v:

$$v = \frac{\left(s^2_{H'_1} + s^2_{H'_2}\right)^2}{\frac{\left(s^2_{H'_1}\right)^2}{n_1} + \frac{\left(s^2_{H'_2}\right)^2}{n_2}}$$

H'_1 = species diversity index in Kg Serasot

H'_2 = species diversity index in Kg Bung Jagoi

f_i = number/individual frequency for the i-th species

$s_{H'_1 - H'_2}$ = differences in standard deviation between Kg Serasot and Kg Bung Jagoi

$n_1 - n_2$ = total number of individuals in Kg Serasot and Kg Bung Jagoi

H_0: There is no significant difference the diversity of anuran amphibians between the upper part and the base of Gunung Jagoi.

H^A: There are significant difference between the diversity of anuran amphibian between the upper part and the base of Gunung Jagoi.

If $p_{calculated} < p_{critical}$, this means that the null hypothesis (H_0), is rejected. Hence the diversity of anuran in Kg Serasot and Kg Bung Jagoi has a significant difference. If $p_{calculated} > p_{critical}$, this means that the null hypothesis (H_0) is accepted, hence diversity of anuran in Kg Serasot and Kg Bung Jagoi is not significantly different.

Mann–Whitney Rank Sum test was used to compare the distribution of the potential available prey in both Kg Serasot and Kg Jagoi. This test is used because the total samples of potential prey in Kg Bung Jagoi are more than the total samples of prey in Kg Serasot. It is a non-parametric test.

Formula:

$$U_1 = R_1 - \frac{n_1(n_1+1)}{2}$$

U1= mean of the sample in Kg Serasot

n_1 = sample size for sample in Kg Serasot

R_1 = sum of the ranks in sample of Kg Serasot

$$U_2 = R_2 - \frac{n_2(n_2+1)}{2}.$$

U_2 =mean of the sample in Kg Bung Jagoi

$n2$= sample size for sample in Kg Bung Jagoi

$R2$=sum of the ranks in the sample of Kg Bung Jagoi

The smaller value of U_1 and U_2 is the one used when consulting significance tables. The sum of the two values is given by

$$U_1 + U_2 = R_1 - \frac{n_1(n_1+1)}{2} + R_2 - \frac{n_2(n_2+1)}{2}.$$

The hypothesis is test whether:

Ho= the distribution of insects both sites are equal

Ha= the distribution of insects in both sites are not equal

To describe the importance of each item consumed, the index of relative importance (IRIt) (Pinkas et al., 1971) was calculated as:

IRIt = (POt) x (PIt + PVt)

IRIt = index of relative importance of the prey items consumed

POt= percentage of occurrence in the diet of the individuals (100 x number of individuals percentage of occurrence / total number of individuals)

PIt= percentage of individuals in the diet of the frogs (100 x total number of t in all individuals/ total number of individuals of all taxa in all individuals)

PVt= percentage of volume of a species in the diet of frog (100 x total volume of individuals of t in all individuals/ total volume of all taxa in all individuals)

Regression between prey in the stomach of frogs and potential prey in the environment and the regression between the prey length (minimum and maximum of each prey) and predator size (SVL) were made using minitab version 13.20. The r^2 value in the regression indicates the strength of the regression. Value +1 or -1 indicates a strong linear association. A value close to 0 indicates a weak association.

H_0: There is no significant difference between prey abundance and diet of frogs of Gunung Jagoi.

H^A: There are significant difference between prey abundance and diet of frogs of Gunung Jagoi.

H_0: There is no significant relationship between frog SVL and prey length it may consume.

H^A: There are significant relationship between frog SVL and prey length it may consume.

If $p_{calculated} < p_{critical}$, this means that the null hypothesis (H_0), is rejected.

If $p_{calculated} > p_{critical}$, this means that the null hypothesis (H_0) is accepted.