DIET, MICROHABITAT AND BIOACOUSTICS OF *Ansonia latidisca* (Amphibia: Anura: Bufonidae), THE BORNEAN RAINBOW TOAD AT GUNUNG PENRISSEN, SARAWAK, MALAYSIA

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Declaration

I hereby declare that the work entitled, "Diet, microhabitat and bioacoustics of *Ansonia latidisca*, the Bornean rainbow toad at Gunung Penrissen, Sarawak, Malaysia" is my original work. I have not copied from any other students' work or from any sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person. I also declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

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(Ong Jia Jet)

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Diet, Microhabitat and Bioacoustics of *Ansonia latidisca*,

the Bornean Rainbow Toad at Gunung Penrissen, Sarawak, Malaysia

Abstract

This thesis presents data on the natural history of *A. latidisca*, a little known bufonid amphibian species, through an investigation of the diet, microhabitat use and bioacoustics of a population at Gunung Penrissen, Sarawak, Malaysia (north-western Borneo). Standard sampling techniques, including visual encounter surveys and spot sampling methods at potential habitats, were adopted.

Dietary data obtained by stomach flushing 46 adults show 12 food categories, dominated by formicids (ants). These results categorize *A. latidisca* as a dietary specialist, with an active preference for feeding on small prey that show clumped distribution. *A. latidisca* is the only known arboreal species of its genus, as identified by this study, and is typically associated with tree trunks, while known congeners are associated with low saplings during the non-breeding season. Patterns of microhabitat utilization shown by the sexes of *A. latidisca* are suggestive of substantial resource sharing. The advertisement calls of male *A. latidisca* consist of two segments. The first segment is usually short (3–5 notes of 5–12 pulses each), and immediately followed by a longer, repeated and uninterrupted note of 112–155 pulses up to 10 seconds. Mean dominant frequencies are around 5.5 kHz, and the fundamental frequency is around 2.7 kHz.

This study has added important baseline knowledge to a poorly known Bornean endemic. The brightly coloured skin, while providing camouflage (specifically, mimicry of mosses and other epiphytic vegetation), may also have aposematic function (specifically, including isolated red pigments on the dorsum of body and limbs). Additional specializations in its

morphology noted include dilated finger tips and relatively elongated body and limbs, features not observed in congeneric species, and considered here as adaptive for an arboreal lifestyle.

Keywords: *Ansonia latidisca*, diet, microhabitat, bioacoustics.

Diet, Mikrohabitat dan Bioakustik *Ansonia latidisca*,

Kodok Pelangi Borneo di Gunung Penrissen, Sarawak, Malaysia

Abstrak

Tesis ini memaparkan maklumat berkaitan dengan sejarah semula jadi sejenis kodok yang kurang dikenali iaitu A. latidisca *dengan menyiasat diet, mikrohabitat dan bioakoustik haiwan tersebut di Gunung Penrissen, Sarawak, Malaysia. Teknik kajian* 'visual encounter survey (VES)' *dan penyampelan secara rawak di kawasan habitat berpotensi telah dijalankan.*

Kepelbagaian diet diperolehi daripada 46 individu menggunakan teknik pemancutan perut menunjukkan 12 kategori makanan dalam perut kodok. Semut adalah diet utama A. latidisca. *Keputusan ini mencadangkan* A. latidisca *sebagai pemakan khas semut dan menunjukkan kecenderungan memilih mangsa bersaiz kecil yang menunjukkan taburan berkelompok. Kajian ini juga menunjukkan* Ansonia latidisca *sebagai spesies kodok pertama yang arboreal di dalam genus* Ansonia *dan habitatnya dikaitkan dengan batang pokok. Kodok* Ansonia *yang lain lebih disekutukan dengan anak pokok yang rendah semasa musim bukan mengawan. Corak penggunaan mikrohabitat oleh kedua-dua jantina* A. latidisca *mencadangkan perkongsian sumber dalam himpunan mikrohabitat. Panggilan* A. latidisca *(jantan) terbahagi kepada dua jenis, satu panggilan pendek diikuti oleh satu panggilan panjang. Panggilan pengenalan adalah pendek (*3–5 *not dan mengandungi 5–12 nadi) and ini diikuti oleh satu not panjang yang diulangi dan tidak terganggu (112–155 nadi) sehingga 10 saat. Min frequensi dominan untuk kedua-dua jenis bunyi adalah sekitar 5.5 kHz dan purata frequensi asas adalah 2.7 kHz.*

Kajian ini telah menyumbang kepada garis tapak sejenis kodok endemik Borneo yang kurang dikenali. Kulit kodok yang berwarna terang berfungsi sebagai penyamaran (iaitu penyamaran sebagai lumut dan tumbuhan epifit) dan mungkin bersifat aposematik (terutamanya pigmen merah pada permukaan kulit dan anggota badan). Pengkhususan tambahan ke atas morfologi kodok ini termasuk dilasi pada hujung jari dan saiz badan dan lengan yang panjang, ciri-ciri yang tidak diperhatikan dalam spesies Ansonia *yang lain, dan ini dianggap sebagai adaptasi untuk kehidupan yang arboreal.*

Kata kunci: Ansonia latidisca, *diet, mikrohabitat*

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Genus *Ansonia* Stoliczka, 1870

The genus *Ansonia* includes 26 species of bufonid species that ranging from Myanmar, Thailand, Peninsular Malaysia, Sumatra, Borneo and the Philippines (Grismer, 2006). A total of 12 species have been recorded in Borneo, of which 11 are endemic to the island (Grismer, 2006; Inger and Stuebing, 2005). The genus has undergone substantial radiation within Borneo, showing altitudinal specialization and morphological differentiation in adults as well as in the larvae (Inger et al., 2001).

The IUCN Red List (2012) identifies six Bornean *Ansonia* species as Near Threatened (*A. albomaculata*, *A. hanitschi*, *A. leptopus*, *A. longidigita*, *A. minuta* and *A. spinulifer*), three as Endangered (*A. platysoma*, *A. latidisca* and *A. guibei*), two species as Vulnerable (*A. fuliginea* and *A. torrentis*). None are protected under the CITES (Convention on Internation Trade in Endangered Species of Wild Fauna and Flora, 2012), presumably because of lack of threat from international trade.

Members of the genus *Ansonia* can be differentiated from other confamilial genera, based on the morphology of larvae and of adults. The adults have a relatively smaller and slender body, with the warty skin of a typical member of the Bufonidae (Inger and Stuebing, 2005). Their tadpoles are adapted to life in strong currents in montane forests and also in lowland rainforest (Matsui et al., 2010), except for larval *Ansonia leptopus*, which lives in drifts of dead leaves that accumulate within eddies of streams (Inger, 1992). The flattened and streamlined body (of *A. leptopus*) and sucker-type oral disk of tadpoles are associated with swift, rocky streams (Inger, 1960). Weak subarticular tubercles and lack of parotoid glands also help distinguish the genus *Ansonia* from other bufonid (Inger, 1960).

All *Ansonia* species are relatively small, with a maximum size of 70 mm (*Ansonia longidigita*). Sexual dimorphism among sexes is also noteworthy (Inger and Stuebing, 2005). Females (25–64 mm) are generally longer than males (20–50 mm), and produce pigmentless ova (Inger and Stuebing, 2005).

1.2. Background Information of the Bornean Rainbow Toad (*Ansonia latidisca*)

Under the family Bufonidae, the Bornean Rainbow Toad belongs to the genus *Ansonia*, which, until 2011, was known from three individuals from two locations in north-western Borneo, namely, Gunung Damus, western Kalimantan and Gunung Penrissen, western Sarawak (Pui et al., 2011). The only published literature is the original description by Inger (1966b), who referred to it as a montane species.

Robert F. Inger described the holotype, an adult male (SVL 35 mm), was collected by Johann Gottfried Hallier (1868–1932), a botanical assistant at the Buitenzorg (at present Bogor) Herbarium, in 1966 from the summit of Gunung Damus (Kalimantan, Indonesian), the paratype, a female (SVL 55 mm), taken by Robert Walter Campbell Shelford (1872–1912), entomologist with the Sarawak Museum, from Gunung Penrissen (Sarawak, Malaysia), in addition to a third specimen from the latter locality, collected by Eric Georg Mjöberg (1882–1938), Curator of the Sarawak Museum.

Since the year 1924, this species has not been sighted (Inger et al., 2004). In June 2011, a small expedition funded by Shell Chair and the Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, to the higher elevations (>1,000 m asl) of Gunung Penrissen rediscovered three individuals of *A. latidisca* from three separate mature trees (ca. 2 m above ground) near forest trails (Pui et al., 2011). The report received widespread publicity in the media.

Figure 1.1: An adult female *Ansonia latidisca*, rediscovered in 2011 at Gunung Penrissen, Sarawak (UNIMAS catalogue number OJJ 0011).

The species is considered taxonomically valid (Matsui et al., 2010), and is basal to the clade of *Ansonia*, suggesting its primitive nature (Matsui et al., 2012). In the IUCN Red List of Threatened Species, it is listed as Endangered, as its extent of occurrence of less than 5,000 km^2 and area of occupancy less than 500 km^2, with all individuals known from fewer than five locations, and there is a continuing decline in the extent and quality of its habitat (Inger et al., 2004). It is not listed in any of the Appendices of CITES.

Gunung Penrissen lies outside the protected system area of Sarawak and has a long history of agricultural activities, especially paddy planting. Rubber and pepper are also grown in all except the steepest terrain. In the last decade, to promote eco-tourism and golf-tourism, the Borneo Highlands Resort, a 2,071 hectare resort, was built close to the summit (at ca. 1,000 m asl). The development had removed most of the native vegetation, although environmental effects remain unstudied.

At Gunung Penrissen, prior to the 1900s, much of the original mountain vegetation was already removed by slash-and-burn activities and more recently, by the development of a golf course and resort. The only pristine forests left now are primary highland mixed dipterocarp forest that survive on sharp ridges or fragments within and adjacent to the golf course and resort at ca. 1,000 m asl (Das and Haas, 2011).

1.3 Thesis Objectives

A. latidisca was only recently rediscovered and its ecology has never been studied before. This thesis presents information on the natural history of *A. latidisca*, investigating the diet, microhabitat and bioacoustics, of this little-known species. The information obtained is totally new to science and adds to the limited knowledge of anuran amphibians in Borneo. Data obtained will be pivotal in conservation implications, especially in land-use management, so that further development does not affect the already endangered *A. latidisca*.

1.4 Specific Objectives

The three research objectives of the thesis are as follows:

i. To assess wild diet, and in association with foraging and feeding habits of *A. latidisca*.
ii. To assess spatial utilization and microhabitat preferences of *A. latidisca*.
iii. To describe the advertisement call of *A. latidisca*.

CHAPTER TWO

STUDY AREA AND GENERAL METHODS

2.1 Study Area

Gunung Penrissen is located in western Sarawak, between latitudes N 1.12˚ to N 1.14˚and longitudes E 110.21˚ to E 110.23˚, and forms the natural boundary between Malaysia's Sarawak State and Indonesia's Kalimantan Barat Province (Figure 2.1). It is drained mainly by Sungei Semadang and forms the headwaters of Batang Kayan. The massif is comprised of a matrix of sandstone and karst features, rising to the rugged ridges of the Penrissen range (Wilford and Kho, 1965)

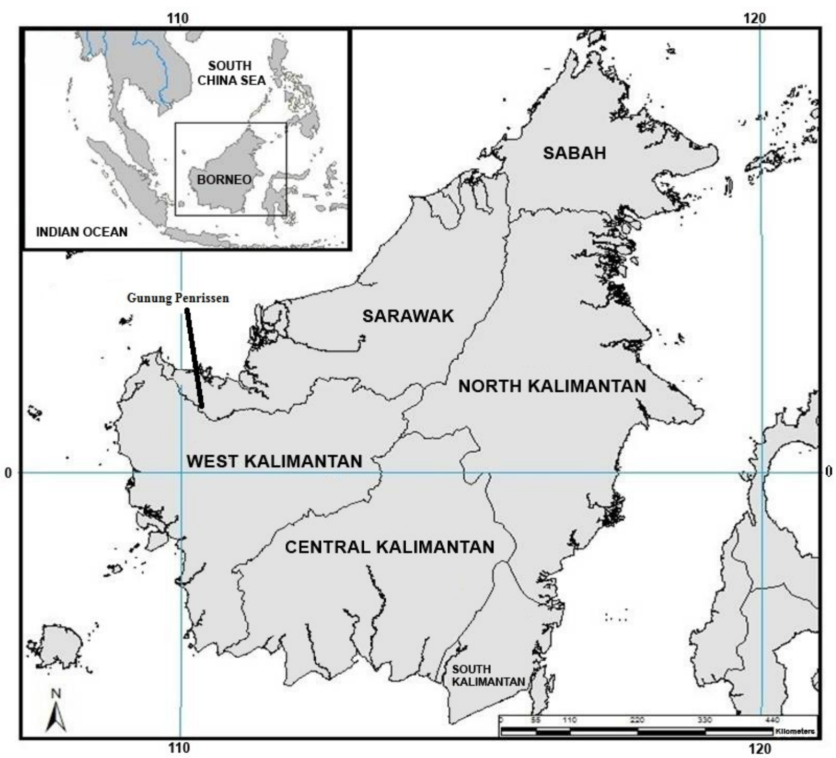


Figure 2.1: Location of Gunung Penrissen in western Sarawak, Malaysia.

2.2 Climate

Western Sarawak experiences two predominant monsoon periods, i.e. the north-east monsoon which prevails from November to March and the south-west monsoon which occurs from June to September (Malaysia Meterological Department, 2012). Inland areas of Sarawak, such as the Penrissen range, generally experience evenly distributed annual rainfall. Slightly less rainfall is received during the period June to August, which corresponds to the prevailing south-westerly winds (Malaysia Meterological Department, 2012). April, May, October are considered non-monsoonal periods. However, these are generalities, and heavy rainstorms can be experienced during the south-west monsoon. Daily temperature fluctuates from 17.5˚C to 25.5˚C in the field.

2.3 Vegetation

The type of forest at the higher elevation of Gunung Penrissen (800–1,300 m asl) is predominantly of primary highland mixed dipterocarp forest. It is a massive forest block, extending to the Kalimantan region of Indonesia. The forest is slightly disturbed on the existing jungle trails on the both sides. Old growth trees are sparsely distributed and along the trails, but understorey plants are very abundant, ranging from seedlings of trees, wild gingers, aroid plants, rattans, shrubs, climbers, ferns and mosses. Since the forest is humid, most of the tree trunks are partially covered by mosses and lichens including the fallen logs on the ground.

2.4 Study Sites

Surveys were completed within designated jungle trails (Figure 2.2). Four transect lines (P, Q, R and S) were established, with each of 500 m transect, except transect line ‘Q’, of 250 m (a landslide that occurred during late February 2012 impeded the extension of the transect).

Figure 2.2: Designated jungle trails at the higher elevation of Gunung Penrissen.

Transect P (999–1,141 m asl; Highland mixed dipterocarp forest, HMDF)

A). It has a relatively flat terrain at the beginning but steep slopes after the first 200 m. A few large-sized dipterocarp trees of large crowns with diameter at breast height (DBH) range from 90–120 cm and height range from 20–40 m was observed. The forest canopy cover is ca. 60–70% and there are two permanent streams within the transect. It has low leaf litter coverage on the ground and average leaf litter depth of ca. 2.5 cm.

Transect Q (900–1,000 m asl; HMDF)

A relatively flat area within the first 200 m of the transect line, with steep slopes until the end. The forest canopy cover is about 70–85% and not many large sized trees were observed long the trails, and mostly comprise *Shorea* sp. of the Dipterocarpaceae family. The leaf litter on the ground is high, of average litter depth 6 cm. The low number of understory plants observed along the trail could be due to the steep slopes.

Transect R (1,146–1,229 m asl; HMDF)

The forest type gradually changes to intermediate mossy forest at an altitude of 1,100 m asl. Forest canopy cover was estimated between 60% to 85% and there is a high percentage of leaf litter on the ground and average litter depth is up to 6.5 cm. It has an abundance of understory plants, ranging from seedling of trees, shrubs, grasses, ferns and fern allies, *Pandanus* species, palms, wild gingers, mosses and lichens and pitcher plants. At the highest elevation within the transect line, trees are prone to lighting strikes.

Transect S (1,146–1,204 m asl; HMDF)

Transect S consists of high number of small sized trees more than 5 cm in DBH is recorded in the transect line. A few medium-sized trees are recorded and range from 30–45 cm and 15–20 m in height. The forest canopy cover is ca. 65–80%. Relatively high leaf litter layer on the ground with average litter depth 6.5 cm and high number of understory plants was recorded. Seedlings of trees, wild gingers, mosses and lichens, climbers, creepers, aroid plants and rattans are in abundance.

2.5 General Methods

2.5.1 Field Sampling

Field work was done at least once every month throughout the year 2012, beginning in January and ended in December. A total of 16 field trips were made and 42 nights were spent in the highland forests. Standard sampling techniques followed include visual encounter surveys (Heyer et al., 1994). By walking slowly using headlight at night, surveys were conducted from 1800–2400 hours. Addition surveys were also carried out at other potential habitats to collect additional data and cover wider range of habitats at different altitudes (particularly 800–1,300 m), the known elevational range for the species.

2.5.2 Data Collection

Each individual of *Ansonia latidisca* caught was marked by fixing a narrow, coloured strip of balloon around its waist before being released at the point of capture (Kunte, 2004; Appendix 1).

The following data were taken for every individual encountered: measurements (including standard length snout-vent length (SVL) and mouth width (MW), using a Mitutoyo™ vernier caliper); sex (male, female or juvenile); and dietary contents by stomach flushing methods. Each individual was weighed to the nearest 0.1 gm, using a 10 gm Pesola™ spring balance.

Sexes of adult toads were determined by the presence of single median external vocal sacs, restricted to adult males that additionally, were smaller than adult females (unpubl. observ.). Individuals of indeterminable sex (typically, the smaller size–classes) were categorized as juveniles. Secondary sexual dimorphism in body size exists in *A. latidisca*, as in the majority of anuran amphibians females are larger than males. Methods specific to subjects discussed in subsequent chapters are discussed under respective topics.

Table 2.1 reviews a list of other frog and toad species sharing the same general habitat at upper Gunung Penrissen found during the study period.

Table 2.1: Checklist of the frogs and toads of upper Gunung Penrissen found during 2012.

ORDER ANURA
Family Bufonidae
Ansonia minuta Inger, 1960
Ansonia spinulifer (Mocquard, 1890)
Leptophryne borbonica (Tschudi, 1838)
Pelophryne spp.
Family Dicroglossidae
Ingerana rajae Iskandar, Bickford and Arifin, 2011
Limnonectes kuhlii (Tschudi, 1838)
Family Megophryidae
Leptobrachella spp.
Leptolalax spp.
Megophrys nasuta (Schlegel, 1858)
Family Microhylidae
Calluela spp.
Kalophrynus spp.
Family Ranidae
Meristogenys spp.
Staurois guttatus Günther, 1858
Family Rhacophoridae
Feihyla kajau (Dring, 1984)
Philautus hosii (Boulenger, 1895)
Philautus petersi (Boulenger, 1900)
Philautus mjobergi Smith, 1925
Philautus spp.
Philautus tectus Dring, 1987

CHAPTER 3

DIET OF *Ansonia latidisca*

3.1 Introduction

Understanding dietary requirements is fundamental in learning amphibian life history, population fluctuations over time and effects of habitat modification (Anderson et al., 1999). Daly et al. (1994; 2007) suggested that toxic frogs obtain toxic substances through exogenous sources by sequestering alkaloids from arthropod prey, with mites and ants contributing most of the alkaloids. Toft (1980a) classified these organisms into two guilds: ant specialist and non-ant specialist, based on their dietary pattern. It is thus of theoretical interest to ascertain the dietary composition, foraging and feeding habits of *A. latidisca*, especially whether, it belongs to the “ant specialist” or the “non-ant specialist” guild.

It is hyphothesized that *A. latidisca* belongs to the ant specialists group. Comparable research conducted in the New World (including Panama, Costa Rica and Peru) has showed that Neotropical bufonids tend to be ant specialists, eating relatively few types of prey other than ants (although some eat small mites as well; Toft, 1980a). On the contrary, non-ant specialists tend to eat a wide variety of prey taxa (Toft, 1980a; Lieberman, 1986; Parmelee, 1999).

3.2 Literature Review

Anuran amphibians are traditionally described as generalist predators, with opportunistic foraging behaviour (Santos et al., 2004). A majority of the invertebrates reported in adult frog diets are arthropods, including arachnids, crustaceans, orthopterans, coleopterans, and especially, ants and mites (Cogălnicueanu et al., 2000; Hirai and Matsui, 2001; Kovacs et al., 2010; Maneyro et al., 2004; Ogoanah and Uchedike, 2010; Toft, 1980a, 1980b; Van Sluys et al., 2006). Small vertebrates, such as fish and frogs, may be occasionally eaten, especially by large growing species (Santos et al., 2004), and cannibalism has also been documented (Inger, 1966a; Ogoanah and Uchedike, 2010). Plant material and stones may sometimes occur in stomach samples in small amounts, and are probably taken incidental to food (Berry, 1966).

Recent studies have suggested otherwise. Taking into account the number of prey items consumed and the contribution of each prey item to the overall diet, anuran amphibians can now be organized in a feeding behaviour gradient, from generalist to specialist (da Rosa et al., 2002). Many species have showed some degree of diet specialization and recognized as specialist feeders are often associated to high proportions of a particular food from the food spectrum available (Toft, 1980a; Santos et al., 2004).

There are two dietary patterns in tropical anurans: the “ant specialists”, which tends to be toxic to potential predators and active searchers of prey, taking many small prey that usually show a clumped distribution, such as ants and termites, and the “non-ant specialists or generalists”, that are cryptic, sit-and-wait foragers, which take relatively few, but larger prey types (Toft, 1980a).

Ants and other small insects make up the diet of probably all *Ansonia* species (Inger and Stuebing, 2005). Hymenoptera was consistently found in stomach of bufonids in the Gunung Mulu National Park, Sarawak (Pui, 2011). In captivity, selective alkaloids fed to poison arrow (or dart) frogs (*Dendrobates*, *Phyllobates* and *Epipedobates*) are readily accumulated into the skin (Daly et al., 2004). However, the natures of noxious substances remain unknown in many cases (Daly et al., 1994; 2004).

See summarized in Table 3.1 on the dietary profile of Bornean *Ansonia* species.

Table 3.1: Summary of literature on the dietary profile of the Bornean *Ansonia* species.

Species	Diet	Reference
Probably all *Ansonia* species	Ants and other small insects	Inger and Stuebing (2005)
A. minuta	Hymenoptera: formicidae (94.4% of the stomach samples)	Direp et al. (2009)
A. platysoma	Hymeoptera (n.s.)	Pui (2011)
A. longidigita	Hymenoptera (DEF = 3.6), Coleoptera (DEF = 0.41), Dictyoptera (DEF = 0.08), Isoptera (DEF = 0.33), Hemiptera (DEF = 0.17)	Pui (2011)
A. torrentis	Hymeoptera (n.s.)	Pui (2011)
A. hanitschi	Hymenoptera (DEF = 3.4), Coleoptera (DEF = 0.64), Dictyoptera (DEF = 0.68), insect larvae (DEF = 0.34), Araneae (DEF = 0.24), Hemiptera (DEF = 0.08), Isopoda (DEF = 0.16), Acari (DEF = 0.04)	Pui (2011)

Note 1: DEF is Degree Food Preference, ranking from 1 to 4 with "4"when only one food item found in stomach. When stomach contained more than one group, the value "3"was given to the most abundant, while the value "2"was given to the second most common and the value "1"was attributed to less abundant groups.
Note 2: n.s is not stated.

3.3 Methods

Stomach flushing method was modified from the protocol of Mahan and Johnson (2007). A 10 ml syringe was loaded with clean water and injected into the toad's stomach via a plastic duct. Stomach content was flushed onto a glass petri dish. Upon completion of stomach flushing, the stomach contents were stored in plastic numbered vials and preserved in 70% ethanol (Lima et al., 2010). In the laboratory, samples were spread onto a petri dish and examined under low magnification using an Olympus™ SZX9 dissecting microscope, to the ordinal level of classification. Parts of unidentified insect are categorized as 'unknown arthropods' and organic material refers to plants that were presumably consumed incidentally. The size of fragmented specimens was estimated based on other specimens with intact bodies. Total volume of food ingested by each individual was estimated by the displacement of water in a graduated cylinder (Das, 1995).

Descriptive analysis of data pertaining to contents of stomach samples were expressed in (Hirai and Matsui, 2000):

Percent frequency of occurrence (F) =

$$\frac{\text{Number of targeted individual in which a given food item was found x 100}}{\text{Number of individuals that provided dietary samples}}$$

Thereafter, food groups were classified as primary when registered in > 50% of stomachs, secondary when between 25 and 50%, and tertiary when under 25% of stomachs following the recommendations of Santos et al. (2004). Non-Metric Dimensional Scale (NMDS) was also included to support the findings above using with IBM SPSS™ Statistics 20.

Individual frog prey dimensions were averaged to calculate mean prey length (APL) and total stomach content volume (Woodhead et al., 2007). Correlation and linear regressions were performed after log transformation of data.

The Berger-Parker diversity index was used to see the difference in the degree of dominance of food types in adult stomach samples throughout the sampling period:

$$d = N_{max} / N$$

where, N is the total number of individuals and N_{max}, the number of individuals in the most abundant resource type. The reciprocal form of the measure was used, whereby the index increases with increasing prey diversity and a decrease in dominance (Magurran, 2004).

For seasonal analysis, data were grouped in two periods: rainy (January–March and November–December) and dry (April–October), although some precipitation was encountered year round during this study.

A manual tracking device was used on one female *A. latidisca* (field number: BH24) to investigate its overnight orientation. The tracking device, described by Shakhparonov and Ogurtsov (2008), consisted of a sewing machine bobbin, with 60 m of cotton thread, held in place on the scapular region of the toad by a harness (Figure 3.1).

Figure 3.1: A female *Ansonia latidisca* (field number: BH24) with a sewing machine bobbin held in place on the scapular region by a harness made of plastic paper fixed by two narrow strips of a balloon.

3.4 Results

Individuals of *A. latidisca* were found throughout year 2012. However, no female was found after September 2012 along designated transects, coinciding with the rainy season. Figure 3.2 shows that monthly variation in numbers of *A. latidisca* encountered during the study period, that were stomach-flushed.

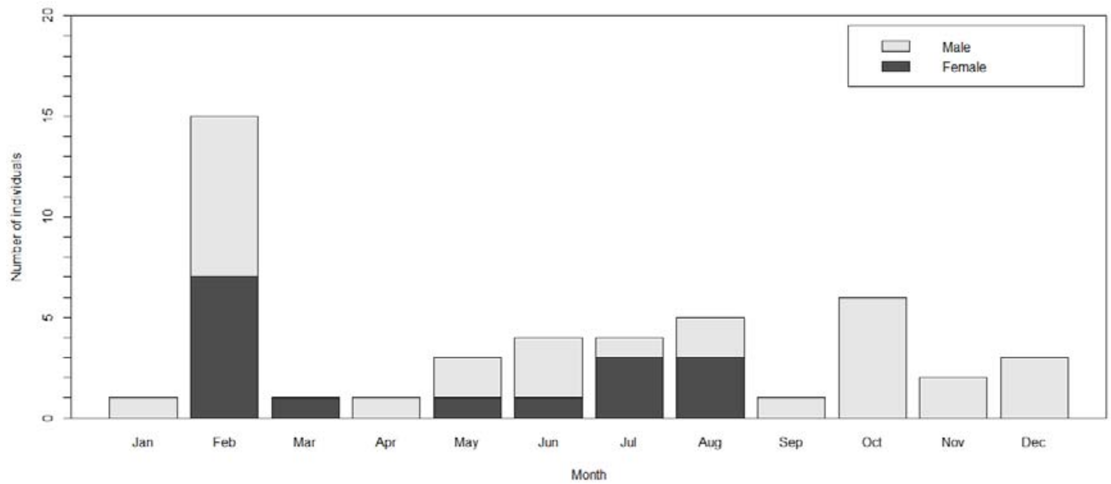


Figure 3.2: Monthly variation in male and female *Ansonia latidisca* encountered from January to December 2012.

3.4.1 Dietary description

A total of 46 adult *A. latidisca* individuals (30 males and 16 females) were examined for their stomach contents. Of these, 93.48% (43 individuals; 28 males and 15 females) had food items in their stomachs. Three individuals with empty stomachs are not included in the dietary analyses. Six of these were recaptured and stomach-flushed at least once. The number of prey items (*t*-test, $df = 10$, $t = 0.793$, $p > 0.05$,) and average stomach content volume (*t*-test, $t = 0$, $p > 0.05$) were also found to have no significant difference in the stomachs of recaptured toads of both sexes. Consequently, all further calculations included first-time and recaptured toads, as no marked difference was indicated in their dietary pattern between captures.

Twelve categories of food were identified to ordinal level of classification and a total of 582 food items were recovered. Two food categories were from the class Arachnida (Araneae and Acarina), and six categories of Insecta (Blattodea, Coleoptera, Diplopoda, Hymenoptera, Isoptera and Thysanura) were observed in the stomachs of *A. latidisca*. The remaining food substances were plant remnants (assumed to be incidentally ingested) and indeterminate insects and their larvae.

Table 3.2 shows that formicids formed the primary food source, and the most commonly found stomach item. It comprised 78.69% by percentage of abundance, of the total diet and showed a frequency of occurrence of 90.70%. Coleopterans (beetles) were found to be the secondary food source (F = 48.84%), and plants were considered as incidental ingestion (F = 23.26%), presumably taken inadvertently while eating other food substances (Berry, 1966).

Table 3.2: Percentage of abundance (A) and percentage of frequency of occurrence (F) and the number of prey individuals per taxon (*N*) in *Ansonia latidisca*.

Taxa	*N*	A (%)	F (%)	Prey Preference
Araneae	4	0.69	4.65	Tertiary
Acarina	1	0.17	2.33	Tertiary
Blattodea	1	0.17	2.33	Tertiary
Coleoptera	34	5.85	48.84	Secondary
Diplopoda	1	0.17	2.33	Tertiary
Diptera	3	0.51	6.98	Tertiary
Hymenoptera: Formicidae	458	78.69	90.70	Primary
Isoptera	43	7.39	13.95	Tertiary
Indeterminate insect larva	1	0.17	2.33	Tertiary
Indeterminate insect	4	0.69	4.65	Tertiary
Thysanura	5	0.86	4.65	Tertiary
Plant material	27	4.64	23.26	Incidental
Total	582	100	-	

There is a significance difference in prey number between the sexes (Table 3.3; Mann-Whitney U test, $p < 0.05$). Stomachs of females contained more food ($N = 418$), volume food range 0.05–1.5 ml; mean prey item per stomach 22.80 ± S.E. 8.87; $n = 15$ than did stomachs of males ($N = 164$) range 0.05–0.3 ml; mean prey item per stomach 5.86 ± S.E. 0.75; $n = 28$.

Table 3.3: Percentage of abundance (A) and percentage of frequency of occurrence (F) and the number of prey individuals per taxon (*N*) in males and females of *Ansonia latidisca*.

	Male			Female		
Taxa	*N*	A (%)	F (%)	*N*	A (%)	F (%)
Araneae	0	0	0	4	0.96	13.33
Acarina	1	0.61	3.57	0	0	0
Blattodea	1	0.61	3.57	0	0	0
Coleoptera	25	15.24	50.00	9	2.15	46.67
Chilopoda	0	0	0	1	0.24	6.67
Diptera	2	1.22	7.14	1	0.24	6.67
Hymenoptera: Formicidae	111	67.68	82.14	347	83.01	86.67
Isoptera	9	5.49	10.71	34	8.13	20.00
Indeterminate insect larva	1	0.61	3.57	0	0	0
Indeterminate insect	4	2.44	7.14	0	0	0
Thysanura	0	0	0	5	1.20	13.33
Plant material	10	6.10	21.43	17	4.07	40.00
Total	164	100	-	418	100	-

3.4.2 NMDS

Two dimensions were derived to discriminate the relationship of different food categories for *A. latidisca* using nearest scaling with low stresses (Stress-1=0.021, Stress-2=0.025, S-stress=0.0003) and high coefficient (Tucker's coefficient of congruence=0.999). One positive loading (Table 3.4 and Figure 3.2a) of dimension 1 indicated that only one variable (Hymenoptera) is useful to be discriminated as the important diet. Dimension 2, on the other hand indicated two positive loadings, of hymenoptera and plant, as shown in Table 3.4. As dimension 1 and 2 show high positive value, 2.214 and 0.336, respectively, only one out of the thirteen food categories was found to be meaningful. This result augments the hypothesis that hymenopterans are the primary source of diet in the stomach of *A. latidisca* sampled.

Table 3.4: Final coordinates of two dimensions for different food categories in the stomach of *A. latidisca* sampled. FCD1 (Final coordinate dimension 1); FCD2 (Final coordinate dimension 2).

No.	Food category	FCD1	FCD2
1	Araneae	-.200	.000
2	Acarina	-.208	-.026
3	Blattodea	-.214	-.026
4	Coleoptera	-.184	-.134
5	Diplopoda	-.217	-.031
6	Diptera	-.204	-.038
7	Hymenoptera	**2.214**	**0.336**
8	Isoptera	-.180	-0.004
9	Indeterminate insect larva	-.209	-.031
10	Indeterminate insect	-.220	-.054
11	Thysanura	-.203	-.069
12	Plant	-.174	**.077**
13	Araneae	-.200	.000

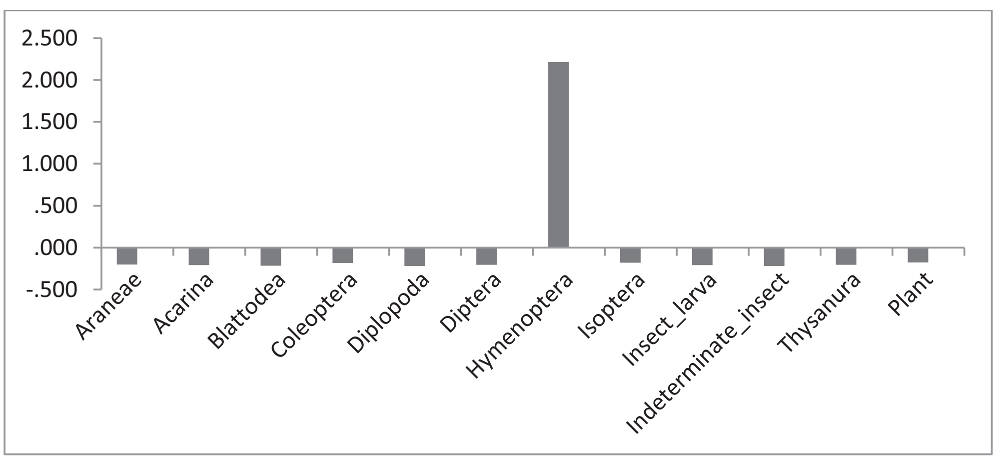


Figure 3.3a: Final coordinate dimension 1 of food categories found in the stomach of *A. latidisca*.

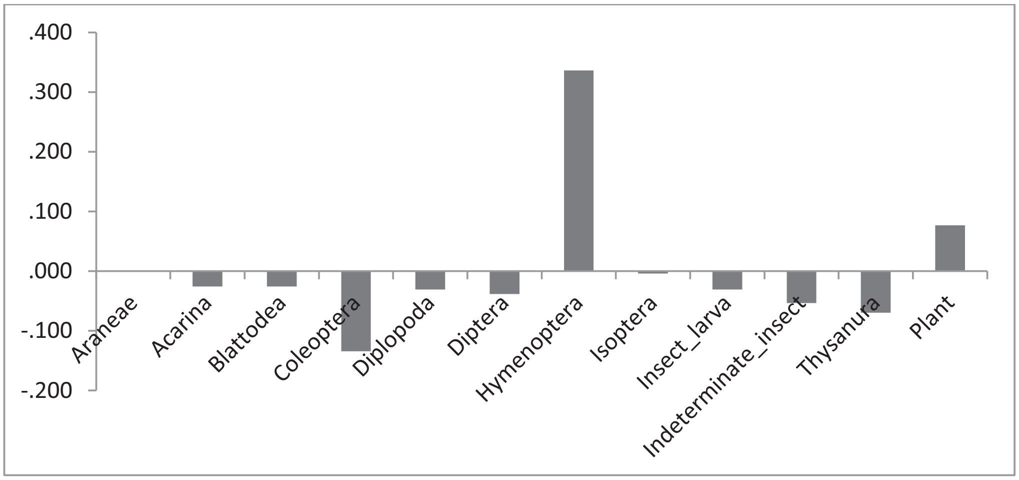


Figure 3.3b: Final coordinate dimension 2 of food categories found in the stomach of *A. latidisca*.

3.4.3 Monthly Diet Variation Cycle

The volume of food ingested by adults (Figure 3.3) did not vary by month (one-way ANOVA, $F_{11, 32} = 1.053$, $p > 0.05$), and there was no significant difference between rainy and dry periods ($\chi^2 = 0.659$, $df = 1$, $p > 0.05$). The mean longest prey was recovered from a female stomach in March, during the wet season. The prey sample size from March, incidentally, was small ($n = 2$).

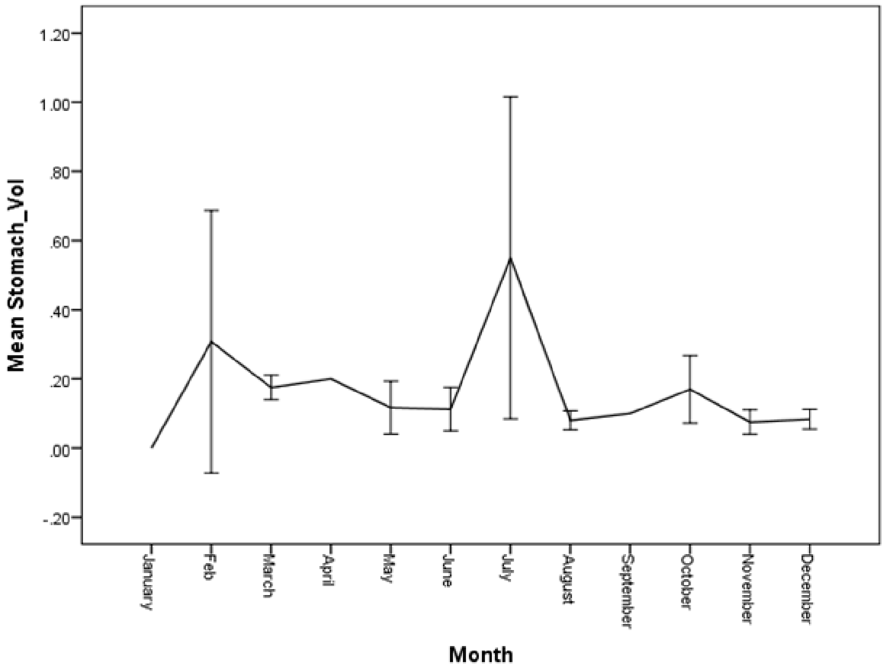


Figure 3.4: Monthly variation in mean stomach volume of prey in stomachs of adult *Ansonia latidisca* with standard deviation.

Monthly Berger-Parker index (Figure 3.4) indices of adults show the greatest dominance values (0.53) during February, that may relate to the fact that the highest number of *A. latidisca* (n = 15) was found during this period. There was a strong positive correlation between the dominance index and number of individuals found in a particular month (Pearson $r = 0.750$; $p < 0.01$), indicating the increasing stomach prey diversity in the higher the number of individuals with stomach flushed. The dominance index between dry and wet periods (t-test, $df = 10$, $t = 0.122$, $p > 0.05$), on the other hand, show no significant difference, indicating that seasonal variation does not affect the dietary components of *A. latidisca*.

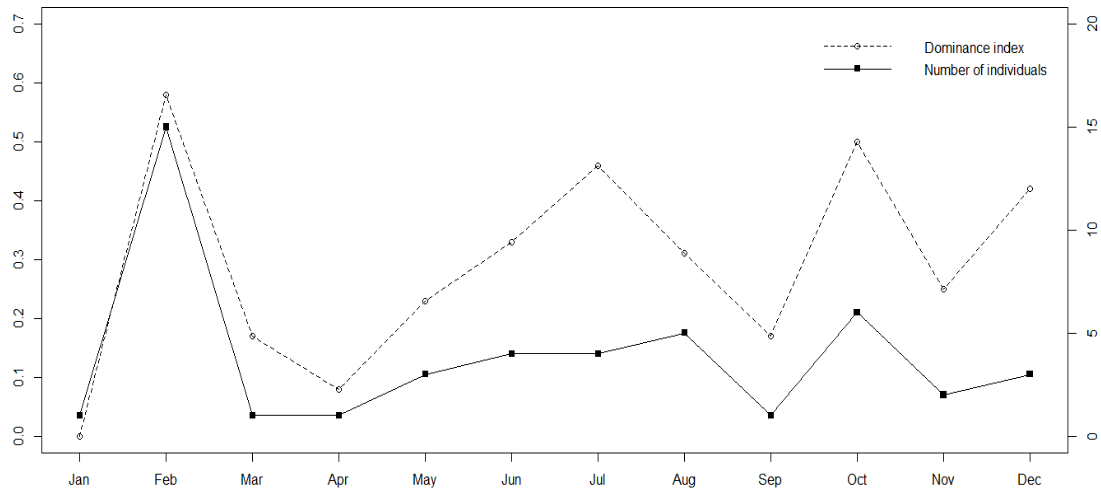
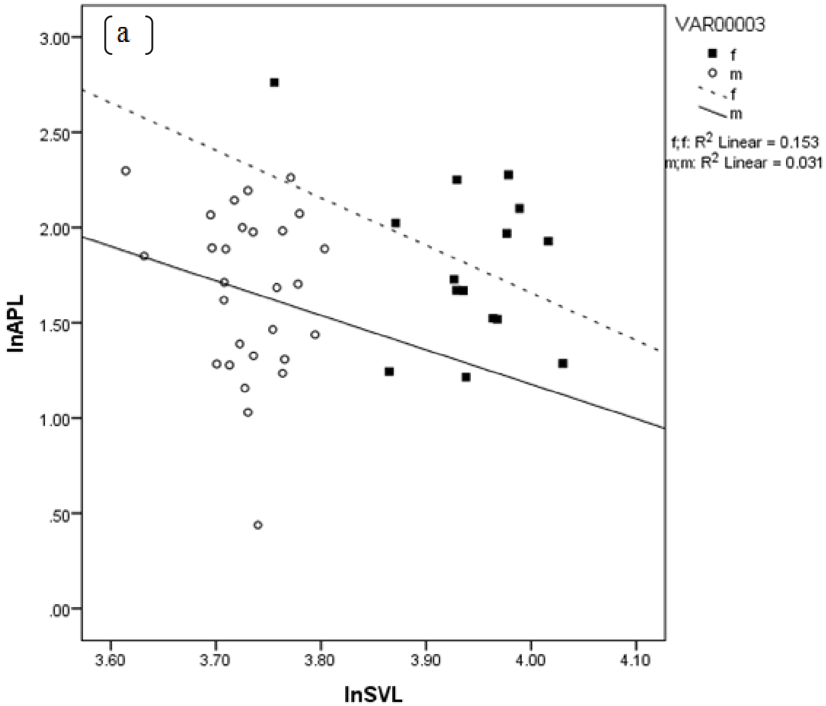
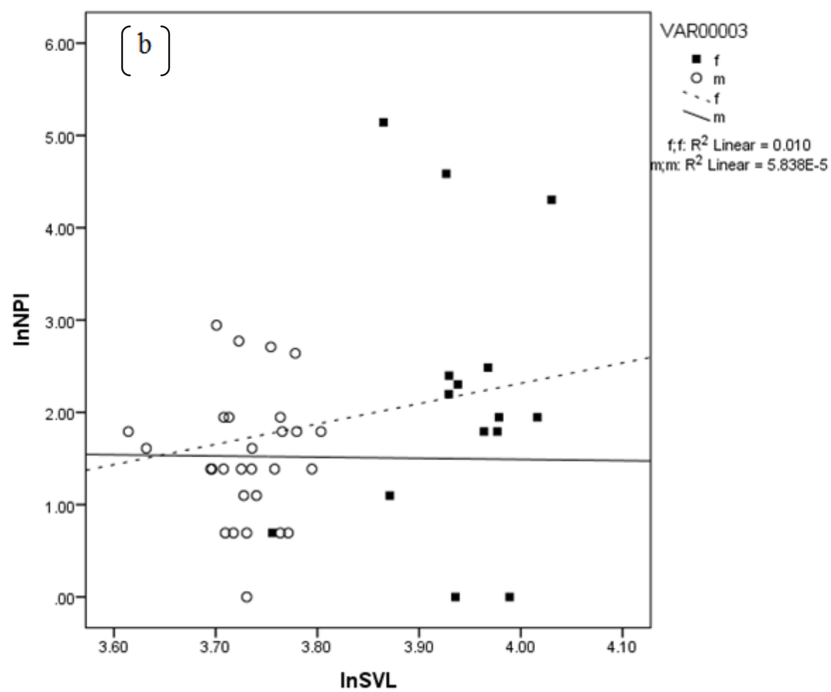


Figure 3.5: Monthly variation in the composition of diet of adult *Ansonia latidisca*, as determined by the inverse of the Berger-Parker index of dominance.

3.4.4 Prey Use in Relation to Sex and Size

The relationships between average prey length (APL), number of prey items (NPI) and snout-vent length (SVL) in the two sexes of *A. latidisca* were tested after log transformation (Figure 3.4a, b and c). The data show no obvious trend for larger individuals to consume larger prey (Fig. 3.4a). Only females show increase in lnNPI consumed with a corresponding increase in body size (here, lnSVL) (Fig. 3.4b), indicating that their bigger body size allow them ingest more prey items than the males. lnAPL and lnNPI were negatively correlated (Pearson r = –0.318), both when considering males and females together ($p < 0.05$), and for the female data alone ($p < 0.05$), indicating that individuals that consumed larger prey had on average consumed fewer prey items (Fig. 3.4c). The largest prey item (prey length 19.43 mm) recovered was the only centipede of the class Chilopoda, found in a stomach of a female (Field ID: BH 14; SVL 42.76 mm).


a
VAR00003
f
m
f
m
f;f: R2 Linear = 0.153
m;m: R2 Linear = 0.031
lnAPL
lnSVL
3.00
2.50
2.00
1.50
1.00
.50
.00
3.60
3.70
3.80
3.90
4.00
4.10


b
VAR00003
f
m
f
m
f;f: R2 Linear = 0.010
m;m: R2 Linear = 5.838E-5
lnNPI
lnSVL
6.00
5.00
4.00
3.00
2.00
1.00
.00
3.60
3.70
3.80
3.90
4.00
4.10

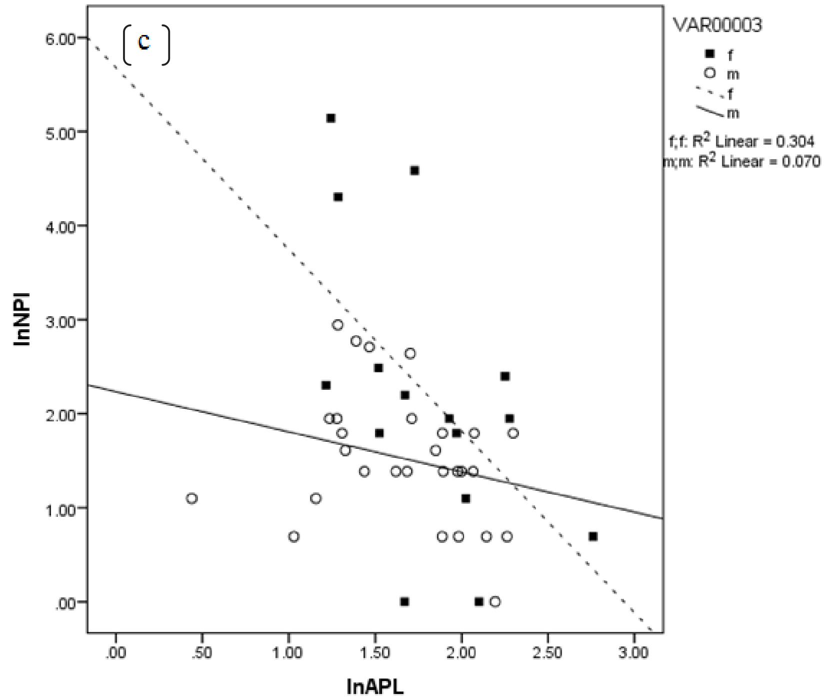


Figure 3.6: Relationship between average prey length (APL), number of prey items (NPI) and snout-vent length (SVL) in male and female *Ansonia latidisca* (log transformed data). Square symbols and dashed lines are females; round symbols and continuous lines are males. **a**) Relationship between lnAPL and lnSVL. Slopes of regression lines: $y = -2.3559x + 11.0912$, $p = 0.149$, for females; $y = -1.7604x + 8.2326$, $p = 0.368$, for males. The relationship is not significant if male and female data are analyzed together ($p = 0.886$). **b**) Relationship of lnNPI and lnSVL. Slopes of regression lines: $y = 1.8380x - 5.6027$, $p = 0.726$, for females; $y = -0.3485x + 2.8244$, $p = 0.969$. The relationship is insignificant when male and female data are analysed together ($p = 0.066$). **c**) Relationship of lnAPL and lnNPI. Slopes of regression lines: $y = -1.9217x + 5.6576$, $p < 0.05$, for females; $y = -0.4279x + 2.2359$, $p = 0.172$, for males. The relationship is significant if male and female data are analyzed together ($p < 0.05$).

3.5 Discussion

A. latidisca is globally recognized as an Endangered species (International Union for Conservation of Nature, 2012) and its distribution is restricted to the primary mixed dipterocarp forest in the upper part of Penrissen range, in Sarawak and the nearby Sambas region of Kalimantan, in north-western Borneo (Inger, 1966b).

3.5.1 Prey Selection in Adult *A. latidisca*

Males had fewer prey in their guts than females (Table 3.2), and the adult diet predominantly comprised of formicids (ants), as indicated by percentage frequency of occurrence of 90.70%. Coleopterans (beetles) were found to be a secondary diet (48.84%), the rest categorized as tertiary. Plant items (including bryophytes) have a high frequency of occurrence of 23.26%. The best explanation is incidental ingestion of vegetation while foraging for prey, but the idea that metamorphosed amphibians may actually ingest plant resources cannot be dismissed. According to Anderson (1999), plant contents may help in elimination of intestinal parasites, provide roughage to assist in grinding up anthropod exoskeletons or provide nutrients and an additional source of water. Additionally, several species of amphibians are known to be herbivorous, including the frugivorous *Hyla truncata* and the folivorous *Euphlyctis hexadactylus* (see da Silva et al., 1989; Das, 1995).

Optimal foraging theory suggests that larger animals should select large prey and overlook small prey which has nutritional value too low to warrant expenditure of energy in their pursuit and consumption (Schoener, 1979). However the major limitation is that within-species evidence is hard obtain, and may be not applicable to all species. Prey size did not appear to be a function of toad body size and larger toads (especially females) tend to eat more prey. This study is in tandem with that of Woodhead et al. (2007), who studied the

Malagasy poison frog, *Mantella aurantiaca*, but this contrasts with data from other studies which found frogs of greater snout-vent length consuming fewer but larger prey (e.g., Berry, 1966; Das, 1995; Hirai, 2002). In fact, a large portion in the diet of large and small individuals of *A. latidisca* is made up of formicids and coleopterans, prey types showing clumped distribution.

3.5.2 Foraging Behaviour: An Ant Specialist

Findings reveal that formicids formed the majority diet (indicated by high percentage–90.70% in frequency of occurrence) and it was the primary food source of *A. latidisca*, supported by results from NMDS. On the other hand, ranid frogs analyzed in Gunung Mulu National Park by Pui (2011) show opportunistic behaviour, being non ant-specialists, and utilizing a wide spectrum of prey categories and none of these were prominently preferred.

According to prey items attributes, an active capture strategy is proposed for *A. latidisca* as the data suggest that *A. latidisca* exhibits active preference for feeding on ants. It is also possible that high proportion of ants found in *A. latidisca* simply reflects a high abundance of ants in their environment. Ants are one of the important sources of alkaloid that is sequestered into the skin glands of frogs of the family Dendrobatidae and toads of the family Bufonidae (Daly et al., 2004; Daly et al., 2007). The substance is stored in the granular or specialized poison gland of the skin and is used as chemical defences against predators or offer protection from microbial infection. In fact, six classes of frog alkaloids identified are well known to come from ants, and with only one respectively from beetles and millipedes (Saparito et al., 2004).

In addition, the female (field number: BH24; GPS coordination: N1.07.038 E110.13.562) which was tracked for orientation was found moving over 13 m (1,330 cm) overnight. The toad was released at 1630 hours (24 August 2012) and was recaptured the next day at 1700 hour (25 August 2012). From the tree trunk it was released [diameter at breast height (DBH): 13.4 cm], the toad hopped to a bigger tree (DBH: 105.0 cm) and was last found on a tree trunk of DBH measuring 14.6 cm.

Toft (1980a; 1980b) mentioned that active foragers elected ants and sit-and-wait foragers, avoided ants. The result corresponds with these studies and it is within the range for those species, such as dendrobatids and bufonids, which have been described as ant specialists (frequency of occurrence, 52–99%; Toft, 1980a; Pui, 2011).

The foraging behaviour in captivity confirms the above relationship. Observation in the lab show that *A. latidisca* forages constantly, searching actively for prey (formicids) supplied. Unlike the non-ant specialists, sit and wait predators presumably recognize prey at a distance and feed during limited periods (Hirai & Matsui, 2001).

3.5.3 Temporal Variation in Prey

Mean food volume per stomach was correlated to the number of toads captured and had their stomach flushed. The spike in mean food volume during July was on account of inclusion of three female individuals with high food volume contents. The females might be eating more to increase their fat reserves for breeding later in the wet season. In North America, female *Anaxyrus californicus* needs to feed for a minimum of two months in order to develop fat reserves essential to produce a clutch of eggs (Sweet, 1992). The disappearance of females over the months of August to December is suspected to be associated with the breeding season, as they presumably use streamside habitats although no female was spotted along the stream during that time. In fact, other *Ansonia* species have adapted to live in rocky and swift streams, and their tadpoles are characterized by sucker-type oral disk (Inger, 1960).

The climate at the higher elevation of Gunung Penrissen is humid, tropical with little yearly fluctuation and the area is considerably wet throughout the year due to frequent mist. Since formicids are the primary food source and abundant throughout the year (pers. obs.), it explains the lack of seasonal variation in the diet of *A. latidisca*. Toft (1980a) also reported a lack of seasonal change in feeding pattern in leaf litter anurans in the lowland rainforest of Amazonian Perú.

3.6 Conclusions

Data obtained from stomach-flushing 46 adults (30 males and 16 females) show 12 food categories. Formicids formed the majority of the diet, with coleopterans being secondary and other arthropod groups being tertiary. Plants recovered from stomach samples are here assumed to be incidentally ingested. The nature of its diet suggests that *A. latidisca* has an active preference for feeding on prey.

Based on the dietary composition and data validated by using NMDS, *A. latidisca* is indeed an ant specialist, and females ingest more prey than males. There was no relationship between predator length (here, mouth width) and prey length as much of the diet of all size classes of *A. latidisca* consisted of formicids and coleopterans, indicating a narrow food preference.

Dietary diversity as recovered from the diversity index appeared unaffected in the different months of the year. The spike in mean food volume in stomach samples during the month of July and the disappearance of females after August is suspected to be associated with the breeding season over the months of August to December, when they presumably use streamside habitats.

This study, in quantifying feeding ecology, showed dietary specialization of *A. latidisca* in feeding almost exclusively on formicids, suggestive of toxic substance origin from an exogenous source (via food uptake and alkaloid sequestration). The brightly coloured *A.*

latidisca, is presumably sending a clear message to potential predators that they are noxious and therefore non-edible.

CHAPTER 4

MICROHABITAT USE BY *Ansonia latidisca*

4.1 Introduction

Microhabitat use is an important component of anuran behavioural studies, in both larval and postmetamorphic stages (Eterovick et al., 2010). In males, differences in physical characteristic of calling site acoustics can affect their success in maintaining territories or attracting females as sound propagation is affected (Wells and Schwartz, 1982). Females may choose sites that improve their fitness for egg-laying (Resetarits et al., 1989) and tadpoles are sensitive to subtle environmental changes such as desiccation and sudden changes in current velocity in some stream-dwelling species (Haramura, 2007).

Schoener (1974) stated that microhabitat is among one of three most important distribution axes for a niche. In this study, spatial utilization and microhabitat preferences of adult *A. latidisca* were investigated.

4.2 Literature Review

A. latidisca is known from isolated and relictual populations in East Malaysia and Kalimantan Province, Indonesia, on the island of Borneo (Pui et al., 2011). The spatial use of its environment by this endangered species has thus far remained unknown, and this is crucial knowledge for the better understanding of the factors that may influence its distribution. According to Inger and Colwell (1977), tropical anuran generalists, particularly insectivores, will have a broad diet, eating according to availability in the microhabitat they occupy. Therefore, frog diets are often closely associated with microhabitats used.

Inger (1994) mentioned that differential microhabitat use by the same species at different sites need to be determined, along with differences of microhabitat use, as this aspect of species ecology can have profound conservation implications.

Rapid development associated with habitat destruction during the last decade has apparently restricted *A. latidisca* to the remaining upper primary mixed dipterocarp forests, situated at the higher elevations of Gunung Penrissen, above the Borneo Highlands Resort. The paratype of a female *A. latidisca* (Fig. 4.1) was collected in the late 1800s by Robert Walter Campbell Shelford and his colleagues from the Sarawak Museum, from Gunung Penrissen, at an attitude of "2,000 feet" (equivalent to 610 m asl; Gower, pers. comm.). No species were found despite substantial effort to survey the relatively intact forested portions of the lower elevations of Gunung Penrissen (around 1,000 m asl; ca. 50 nights) by other researchers

Figure 4.1: Paratype of female *Ansonia latidisca* [British Museum (Natural History) specimen number 99.12.8.12; SVL 56 mm]. Photo: David Gower.

In this study, data from the upper highland mixed dipterocap forest of Gunung Penrissen, specifically, the Summit Trail at Batu Panggah, are presented. Microhabitat utilization data of *A. latidisca* are also important for conservation agencies interested in land management, and consequently, in species conservation.

4.3 Methods

The position of each captured individual was recorded and data from recaptured individuals were included in the analysis. To estimate niche breadth of males and females of *A. latidisca* during the wet (= rainy) and dry seasons, microhabitats used were classified based on perching substrate (leaf, rock, branch, tree trunk), vertical height (on ground or above ground) and distance from water (0−1 m or > 1 m) with the recommendations by Eterovick and Ferreira (2008). All possible combinations of such features resulted in 12 microhabitat types, of which five were used by the species (see Table 4.1). A non-parametric, chi-square test was employed to see if any significant difference in between the microhabitat use of male and female toads.

Niche breadth was estimated on a Microsoft Excel spreadsheet and niche overlaps using EcoSim (Gotelli and Entsminger, 2004) between males and females and between dry and rainy periods were investigated, to test whether the values were lower than expected by chance, indicating differential spatial distribution between sexes or along time, respectively. Monte Carlo simulation was used to see the possible relationship.

Niche breadth was calculated using Levin's index (Levins, 1968),

$$B = \frac{1}{\sum P_i^2}$$

where B = Levin's measure of niche breadth, and,
Pi = the proportion of individuals recorded from distance class i

To standardize the index above, Levin's standardized measure of niche breadth was calculated using the formula (Levins, 1968):

$$B_A = \frac{B-1}{N-1}$$

where, B_A = Levin's standardized measure of niche breadth,
B = Levin's measure of niche breadth, and,
N = the number of distance classes

Pianka's (1973) overlap index was used with retained niche breadth and reshuffled zero states (RA3 randomization algorithm) and equiprobable resource states in the environment (Gotelli and Entsminger, 2004). The index renders values between 0 and 1 (between complete dissimilarity and complete similarity in resources used, respectively).

The Pianka overlap index (following Pianka, 1973) is:

$$O_{jk} = \frac{\sum P_{ij} P_{ik}}{\sqrt{\sum P_{ij}^2 x \sum P_{ik}^2}}$$

where P_{ij} and P_{ik} are the proportional uses of a microhabitat by j (male) and k (female) *A. latidisca*.

Null model usage for community ecology has been summarized by Schoener (1974) who stated that niche overlap observed in nature is smaller than expected by chance. Thus, it can be determined whether the observed segregation is caused by competition or by stochastic processes.

4.4 Results

4.4.1 Microhabitat Utilization by Adult *A. latidisca*

A total of 46 sightings were recorded, represented by 30 records of males and 16 females. Slightly more *A. latidisca* were sighted during the dry (n = 24) compared to rainy periods (n = 22). Standardized niche breadth values were higher in males than females, with values for both males and females higher in the dry than rainy periods (Table 4.1).

Table 4.1: Microhabitat parameters utilized by male and female *Ansonia latidisca*.

Microhabitats: substrates, height (cm), distance to water (m)	**Males**		**Females**		
	Dry	Rainy	Dry	Rainy	Total
Rock, AG, 0−1	3	1	0	0	4
Rock, OG, 0−1	2	0	0	0	2
Leaf, AG, 0−1	2	3	0	0	5
Leaf, AG, > 1	3	7	2	1	13
Tree trunk, AG, > 1	6	3	6	7	22
Total	16	14	8	8	46
Niche breadth, B	4.13	2.88	1.6	1.28	
Levin's standardized measure of niche breadth, B_A	0.426	0.47	0.15	0.07	

Note: AG = above ground and OG = on ground

Horizontal position here refer to the distance to nearest water source, of which two categories are recognized, 0−1 m or > 1 m. Eleven sightings were recorded near water sources (0−1 m; 11 males and 0 female) and 35 sightings (> 1 m; 19 males and 16 females) were made away from water. The maximum perch height recorded was 7 m above ground and only two male individuals were found on the ground (substrate = rock) along a permanent stream. Only three substrates were utilized by adult *A. latidisca*. Tree trunk was the most used substrate type (22 sightings; nine males and 13 females), followed by leaves (18 sightings- 15 male; three females) and six sightings on rocks (6 males; 0 females). No *A. latidisca* were detected on the ground of bare soil or on leaf litter.

Classification of microhabitat used by unweighted pair-group (UPGMA) method and average Euclidean distance method based on microhabitat preference was shown in Figure 4.2. Two main clusters could be observed; group A (minority) individuals were found perching on rocks and group B1 individuals (majority) were observed either perching on leaves or on tree trunks. Group B1 can be further divided into a subgroup B2, consisting of individuals which were observed perching on leaves, near or away from water (see Appendix 2).

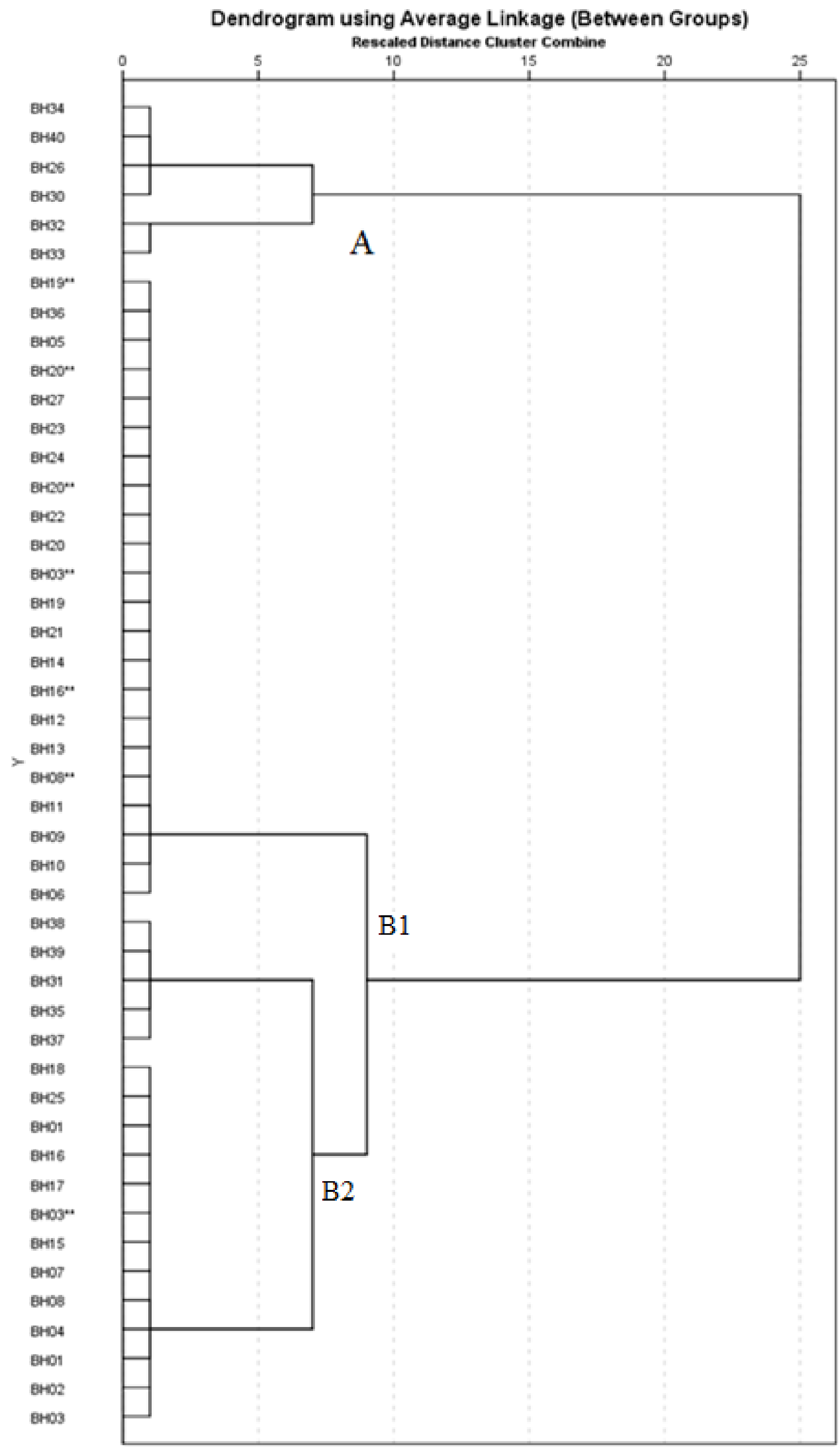


Figure 4.2: Dendrogram using average linkage clustering on unweighted pair-group (UPGMA) analysis and average Euclidean distance method on data based on counts of individual (denoted by BH) microhabitat preference in *A. latidisca* (Note: ** refers to recaptured individuals).

Table 4.2: Spatial utilization (horizontal and vertical positions and substrate type) and niche breadth scores of both males and females of *Ansonia latidisca* collected at the upper reaches of Gunung Penrissen.

	Sample size (n)	Horizontal position			NU	B	B_A	χ^2 p-value	
Ansonia latidisca		0–1 m	>1 m		2				
Male	30	11 **(36.67)**	19 **(63.33)**			1.87	0.87	0.10	NS
Female	16	0 **(0.00)**	16 **(100)**			1.00	0.00	0.02*	S
	χ^2 p-value	0.02*	0.17						
		S	NS						
		Vertical position							
		On ground	Above ground			2			
Male	30	2 **(6.67)**	28 **(93.33)**			1.14	0.14	0.53	NS
Female	16	0 **(0.00)**	16 **(100)**			1.00	0.00	0.39	NS
	χ^2 p-value	0.30	0.83						
		NS	NS						
		Substrate type							
		Rock	Leaf	Tree Trunk	3				
Male	30	8 **(26.67)**	13 **(43.33)**	9 **(30.00)**		2.86	0.93	0.22	NS
Female	16	1 **(6.25)**	3 **(18.75)**	12 **(75.00)**		1.66	0.33	0.06	NS
	χ^2 p-value	0.14	0.18	0.03*					
		NS	NS	S					

Note: * denotes exact significant at $p < 0.05$.
Abbreviations; NU = overall number of microhabitat category utilized, NS = non-specialization, S = specialisation, B = niche breath and B_A = standardized niche breath.

4.4.2 Niche Overlap

Niche overlap value was higher than expected between dry and rainy periods for both males and females as well as between sexes in both periods (Table 4.3), both values were found to be significant (x^2 test; $p < 0.05$).

Table 4.3: Niche overlap values between sexes and between dry and rainy periods as indicated by the Pianka (1973) index.

	Observed mean	**Mean of simulated indices**	**Monte Carlo simulation, *p*-value**
Between sexes	0.73	0.48	**0.04***
Between dry and rainy periods	0.94	0.59	**0.01***

Note: * denotes exact significant at $p < 0.05$.

4.5 Discussion

In the present study, all females *A. latidisca* were found to stay away from water (x^2 test; $p < 0.05$) and both sexes of *A. latidisca* were likely to be associated with tree trunks (x^2 test; $p < 0.05$) within primary mixed dipterocarp forest of Gunung Penrissen. This is the first record of an *Ansonia* species associated with tree trunks (congeneric species are associated with saplings and emergent macrophytes and rocks stream edges). This behavior is in accordance with Matsui et al.'s (2012) description of arboreality in the species.

4.5.1 Spatial Utilisation by *A. latidisca*

4.5.1.1 Horizontal Position

Females showed significant variation in terms of horizontal positioning (x^2 test; $p < 0.05$) and narrow standardized niche breath ($B_A = 0.00$), indicating specific preference of sites away from water. Males otherwise have a wider standardized niche breadth ($B_A = 0.87$), and they could be found in and along swift, rocky streams. At higher altitudes, during precipitation, rain water infiltrates the soil into deep throughflow channels, creating groundwater (Wohl et al., 2012), that may explain the shortage of permanent surface water sources at the survey site.

4.5.1.2 Vertical Position

Chi-square tests show no statistically significant difference for use of ground or aerial microhabitats by the two sexes (x^2 test; $p_{male} = 0.53$; $p_{female} = 0.39$). Only two males were found on the ground (on exposed rock) within torrents of a permanent stream. The rest (n = 44; 28 males and 16 females) were found above ground, from as low as 5 cm, to 7 m above ground.

Males may prefer an elevated position to broadcast their calls, but not high enough to be exposed to wind and less moist conditions. Elevated calling sites may maximize sound transmission distance, which is advantageous for males (Townsend, 1989). Females may choose to hide in a tree-hole for reproduction and reduce predation risk. A female *A. latidisca* (field number: BH24) was briefly tracked to study its movement and orientation over a one-day period. Between the time of first released (1630 hours; 24 August 2012) and recaptured the following day (1700 hours; 25 August 2012), the individual concealed itself in a narrow tree-hole of a dead log (120 cm above ground). The tree-hole opening was covered with a thick growth of mosses and consequently, the hole was initially unnoticed. This presents the only evidence the use of tree holes by the species. Figure 4.3 illustrates the orientation of the female toad during its one-day study of its orientation and movement.

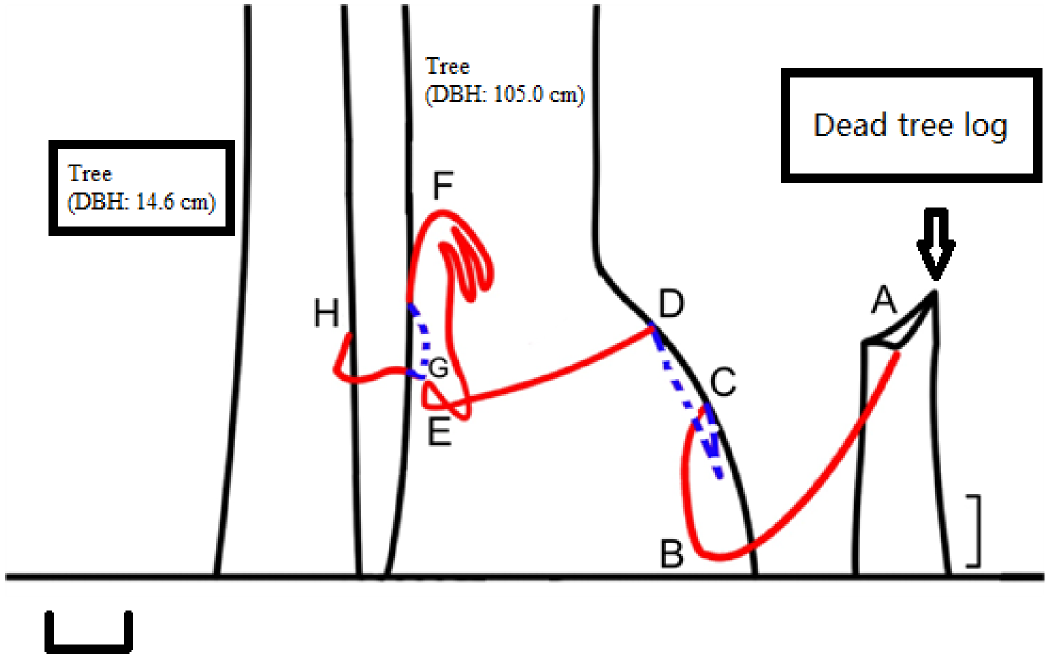


Figure 4.3: Illustration of a female *Ansonia latidisca* (field number: BH24) and its overnight orientation. Continuous red line show visible route and dashed blue line represent inferred route from a frontal view. The individual was released at Point A (1.4 m above ground) on a standing dead tree log (DBH: 13.4 cm), also the site of initial observation. It concealed itself in a narrow tree-hole before it landing on the buttress root of the next tree (DBH: 105.0 cm). It waited around Point C. At Point D (1.7 m above ground), it went around the tree and moved to a position slightly below at Point E (1.3 m above ground). During the 24-hour course of orientation, it rested at Point F (the highest height; 2.3 m above ground) before descending to Point G and jumped to the last tree (DBH: 14.6 cm). It was last found at 1.6 m above ground (Point H), on the trunk of a tree (figure credit: Pui Yong Min; vertical scale bar = 0.5 m and horizontal scale bar = 0.3 m).

4.5.1.3 Substrate Types

Three substrate types were utilized by *A. latidisca*, namely, leaves, rocks and tree trunks. Tree trunk was found to be the most utilized substrate type by both males and females of *A. latidisca* (x^2 test; $p < 0.05$; see Table 4.2).

These are also the first records of the association of an *Ansonia* species with tree trunks. Popularly referred to as stream dwellers, *Ansonia* species are associated with small streams, and on the banks and along fast-flowing streams and rivers, perching on low vegetation in rocky terrain (Dring, 1983; Inger, 1960).

4.5.2 Niche Analyses

Niche breadth was higher in males, with values for both males and females being similar in both wet and dry seasons. Results suggest that males are more generalists in habitat use than females, with regards of higher B_A values within the three microhabitat positioning used, in occupying a wider range of microhabitats (Table 4.1). Males are also likely to be found near water, presumably for finding mates, and females specialize on tree trunks. Females presumably use streamside habitats to breed although none were found during the study period. Males of Bornean ranid frog can be commonly found on riverbanks year round, but females spend most of their time in the forest and only arrive to the river to breed (Iskandar, 2004), and similar observations have been reported for the tree toad, *Pedostibes hosii* by Inger and Stuebing (2005). In terms of vertical position, both males and females are arboreal, as they were consistently found above ground.

Results generated from EcoSim suggest that observed mean niche overlap is significantly greater than expected by chance (Monte Carlo simulation; $p < 0.05$). This is statistically different than the null model under RA3 randomization algorithm, indicating that niche segregations do not exist. The observed large overlap in response to, between sexes and between different time periods, reflects similarity in resource sharing within the microhabitat assemblage and presumed to be due to lack of competition.

4.6 Conclusions

This chapter reports microhabitat use by *A. latidisca*. It is shown to be the first arboreal species in its genus associated with tree trunks, congeners being stream dwellers and frequently associated to tree trunks. Morphological specializations for an arboreal lifestyle include dilated finger tips and relatively elongated body and limbs. Additionally, its skin texture and colour mimic tree trunks that are covered with lichens and moss, presumably affording camouflage.

Patterns of microhabitat utilization shown by the two sexes of *A. latidisca* using data simulated by EcoSim, are suggestive of substantial resource sharing within the microhabitat assemblage. Low population densities may contribute to reduced intraspecific competition.

CHAPTER 5

BIOACOUSTICS OF *Ansonia latidisca*

5.1 Introduction

Vocalizations are an important component of the behaviour of most frogs and toads. Bogert (1960) classified male anuran calls intro five discrete categories based on the context in which they occur: (1) advertisement calls, (2) territorial calls, (3) release calls, (4) distress calls, and (5) warning calls. In this chapter, only the advertisement call will be discussed.

Bogert (1960) used the term 'mating call' to describe the principal signals given by males during breeding season, but the call was soon replaced with 'advertisement calls' due to its multiple functions (Wells, 1977b). Advertisement calls provide information on a male's position relative to those of other males and help maintain distance between calling individuals, and males are more likely to approach or attack speakers playing high-pitched calls of small males, but retreat from the low-pitched calls of larger males during competition (Wells, 1977a; 2007).

Advertisement calls of anuran amphibians may consist of a single note (e.g., *Limnonectes laticeps*; Dring, 1979), a series of identical repeated notes (e.g., *Ansonia hanitschi*; Malkmus et al., 2002), or a long high-pitched trill (e.g., *A. longidigita*; Malksmus et al., 2002). Studies by many authors, including Zainudin et al. (2010), have revealed that advertisement calls can have appropriate characters for species recognition, and methods are cost effective. For instance, the note repetition rate can be a good indicator (Zainudin et al., 2010). Properties of advertisement calls can also serve as indicators of physiological condition and genetic quality of the caller (Welch et al., 1998), and females may thus be using call characteristics and call effort as honest indicators of male fitness (Emerson, 2001). Strong sexual selection by females to discriminate acceptable signal variants is important to avoid heterospecifics because they often do not result in viable offspring (Ryan and Rand, 2001).

A review by Sukumaran et al. (2006) reveal that studies of Bornean frog advertisement call are limited and most call data are abbreviated, onomatopoeic or verbally described. About 65% of frog species have undescribed calls, and lack of sonograms and oscillograms, therefore, do not lend themselves to acoustic analysis. Only 75 species (under 49% of the fauna) have known calls. Sukumaran et al. (2010) subsequently added to this inventory, with call descriptions of additional species.

In this chapter, the advertisement call of three *A. latidisca* individuals is explained.

5.2 Literature Review

Acoustic communication plays a fundamental role in anuran reproduction, and vocalization is by far the best studied anuran communication signal because they are effective for communication at night or in dense vegetation (Hödl and Amézquita, 2001).

Studies have revealed that different syntopic species have different seasonal and nightly pattern of calling activity (Inger and Stuebing 2005). Roy and Elepfandt (1993) found that three abundant Indian frog species emits mating calls after dusk and no calling is recorded if there is no cloud cover or sporadic rainfall during the day.

According to Wells (2001), males of different species not only vary in their overall calling pattern, but also in their rate of calling, the structure of their calls, and call intensity. This pattern has been reported by Zainudin et al. (2011) in several Bornean frogs of the genus *Hylarana* in Sarawak. In a multi-species community, effective communication as such has necessitated modifications in the calls to curb the problem of inter-specific acoustic interference (Duellman and Trueb, 1986). Male frogs react to the call of competing males by increasing calling rate, call duration, call complexity, or the number of notes in a call (Wells, 2001), and female *Hyla versicolor* may use differences in calling performance as indicators of genetic quality of individual males (Welch et al., 1998).

A synopsis of bioacoustics studies of genus *Ansonia* in Borneo is shown in Table 5.1

Table 5.1: Checklist of the genus *Ansonia* from Borneo, annotated with status information on bioacoustics and their sources. Status of description: 0 = unknown; 1 = abbreviated, onomatopoeic or verbal description; 2 = sonogram/oscillogram and analytical description (adapted from Sukumaran et al., 2006; 2010).

	Status of description
A. albomaculata	1 (Inger and Stuebing, 2005)
A. echinata	0
A. fuliginea	0
A. guibei	0
A. hanitschi	1 (Inger and Stuebing, 2005); 2 (Malkmus et al., 2002)
A. latidisca	0
A. leptopus	1 (Inger and Stuebing, 2005); 2 (Inger and Dring, 1988; Sukumaran et al., 2010)
A. longidigita	1 (Inger and Stuebing, 2005); 2 (Inger and Dring, 1988; Malkmus et al., 2002)
A. minuta	0
A. platysoma	1 (Inger and Stuebing, 2005); 2 (Malkmus et al., 2002)
A. spinulifer	2 (Matsui, 1982)
A. torrentis	2 (Dring, 1983)

Table 5.2 is a summary of information available on acoustics recorded from Bornean *Ansonia* species, including analytical descriptions of acoustic properties. Calls of only two montane species: *A. hanitschi* and *A. torrentis* are available. The rest, (*A. fuliginea and A. guibei*) have unknown calls.

Table 5.2: Summary of literature on the bioacoustics of *Ansonia* species from Borneo.

Species	Acoustics Properties	Reference
A. hanitschi	Each call series takes 6−10 seconds and is composed of equal notes with a note repetition rate of 6 per second. Each note (duration 140−150 ms) comprises a group of 3 pulsed single notes (duration 21−50 ms) which are repeated at intervals of 25−35 ms; frequency range 5.4 to 6.0 kHz.	Malkmus et al. (2002)
A. longidigita	The long note series (0.9 to 10.5 seconds) consisting of 4−135 notes at a note repetition rate of 12.5−15.9 per seconds, each note (duration 36−44 ms) consisting of 2−3 pulses (duration 7−33 ms). The internote interval takes 31−36 ms; dominant frequency is 3−4 kHz with a harmonic at 10.8 kHz.	Malkmus et al. (2002)
A. longidigita	Trills of 4−135 notes in 0.31−10.53 seconds, at a rate of 12.50−15.85 notes per seconds; dominant frequency is 3.4−4.0 kHz, with a harmonics at 7.2−7.4 kHz and a frequency modulation (FM) element in each note.	Inger and Dring (1988)
A. leptopus	Short trills of 7−28 notes in 0.30−1.22 seconds, at a rate of 21.88−25.00 notes per seconds; dominant frequency is 2.9−3.4 kHz, with harmonic at 6.1 to 6.4 kHz and no sign of FM.	Inger and Dring (1988)
A. leptopus	Each call consists of five notes over a 0.3 seconds period (duration = 0.04 seconds). Each note consists of a twin-pulse trill, the first pulse of slightly shorter period than the second, at 0.01 seconds, as opposed to 0.02 seconds, and 0.06 seconds between each pulse. Both pulses of each note are generally of the same frequency than the last three (2530 and 2650 Hz as opposed to 2720 Hz). Time between each call varied typically around 2 seconds.	Sukumaran et al. (2010)

Continued…

A. platysoma	The series consists of more than 100 weakly pulsed notes (duration 10−15 ms, internote interval 100−150 ms; the interval between the last two notes of a note series is distinctly longer; note repetition rate 5−7 per seconds) with a frequency of 7.8 to 8.2 kHz.	Malkmus et al. (2002)
A. torrentis	Elements are repeated with a period of 0.13−0.18 seconds. Each call is composed of a strong, poorly tuned note, 0.03 seconds in duration, followed by two or three very weak notes, to form series 0.06−0.09 sec in duration. Dominant frequency is at 5−6 kHz, with energy of the poorly tuned notes spread over the range 4.2−7.0 kHz. FM and, amplitude modulation (AM) are absent.	Dring (1983)
A. spinulifer	a) The short call consists of a single short note (0.01 seconds), which is emitted 2.2 times per seconds. Harmonic are partial and not evident. Dominant frequency is 2.9−3.0 khz, and a less intensive frequency band is sometimes traced at 1.8−2.0 khz. b) The long call sometimes follows the short call. It lasts 0.72−0.74 seconds and consists of six to eight notes. The notes are irregularly emitted and variable in duration (0.01−0.06 seconds). Each note includes one to four pulses. The note basically has clear harmonics, and the fundamental frequency ranges about 1.1 kHz. Dominant frequency range varies within a call, and is lower in the notes at the beginning and at th end of the call. In the notes of the middle portion of the call, the dominant frequency is 3.9 kHz (probably the fourth harmonic), and evident FM are recognized within a note.	Matsui (1982)

5.3 Methodology

Calls were recorded using the bioacoustic techniques described by Dorcas *et al.* (2009). All call recordings were made with a Marantz PMD670 portable solid-state digital recorder, coupled to a Sennheiser ME 66 shotgun microphone attached to a Sennheiser K6P power module. The Marantz solid state recorder was set to save mono-channel audio using the PCM (Pulse Code Modulation) algorithm, at the unit's maximum sample rate of 48 kHz, in the Wave-form audio file format (".wav"). This format allowed subsequent analyses without loss of quality as a result of non-linear compression (as in .mp3 files).

The calling toads were first tracked down before they could be seen visually. The recording apparatus was set up with the microphone pointing directly at the calling individual and placed as close to the toad as possible. After fixing gain level, all headlamps and other artificial illumination sources were switched off, and all movement ceased for the duration of recording. Basal body temperature of calling toads was taken using a Blue Gizmo^{TM} digital pocket thermometer, model BG366.

Once the recording was completed, the calling individual was captured and euthanized using a chlorobutanol solution. Liver tissues were extracted and preserved in 95% ethanol. The specimens were fixed in 4% formalin, preserved in 70% ethanol, and deposited in the museum of the Institute of Biodiversity and Environmental Conservation (IBEC), Universiti Malaysia Sarawak, Kota Samarahan, to serve as vouchers.

Calls were analyzed using Raven Lite (vers. 1.0 for Windows) and SoundRuler (vers. 0.9.6.0) (Gridi-Papp, 2003–2007) after transferring the sound files directly from the flash card media of the recorder. Sonogram and oscillogram of *A. latidisca* were displayed using Raven Lite (vers. 1.0 for Windows), with the recommendation by Sukumaran et al. (2010). The call variables were measured following Matsui (1997), Bee (2003) and Zainudin et al. (2010) (Table 5.3). Pearson correlation test were used to see the relationships between SVL, fundamental frequency and dominant frequency, and note repetition rate with anal temperature of the toad. PCA analysis was performed to determine the best variables in describing the species (Zainudin et al., 2010).

Table 5.3: Call characters measured in this study following Matsui (1997), Bee et al. (2000), and Zainudin *et al.* (2010).

	Group	Variables	Measured Characteristics
1	Calls	Note	A group of pulse
2		Note duration	Time calculated from the beginning of the first pulse to the end of the last pulse in a note
3		Note gap	Time calculated from the beginning of one note to the beginning of the next note
4		Note repetition rate	Number of notes per second
5	Pulses	RelPulsePeak	Pulse maximum amplitude relative to call maximum amplitude
6		PulDur_0	Pulse duration between 0% amplitude marks
7		PulDur_10	Pulse duration between 10% amplitude marks
8		PulDur_50	Pulse duration between 50% amplitude marks
9		PulDur_90	Pulse duration between 90% amplitude marks (sustain)
10		PulOn_90	Pulse time for onset to 90% (attack)
11		PulOn_peak	Pulse time for onset to peak (rise)
12		PulOff_peak	Pulse time for peak to offset (fall)
13		PulOff_90	Pulse time for 90% to offset (decay)
14		PulShapeOn	Pulse shape (10:50% onset / 10:90% onset)
15		PulShapeOff	Pulse shape (50:10% offset / 90:10% offset)
16		PulInter	Pulse interval
17		PulPeriod	Pulse period (time peak to peak)
18		PulDuty	Pulse duration / period (Duty cycle)
19		Crest Factor	Pulse peak / rms
20	Energy	Ener-0-10-Beg	Energy between initial 0:10% peak amplitude
21		Ener-10-50-Beg	Energy between initial 10:50% peak amplitude
22		Ener-50-90-Beg	Energy between initial 50:90% peak amplitude
23		Ener-90-Peak-Beg	Energy between initial 90%: peak amplitude
24		Ener-Peak-90-End	Energy between final peak: 90% amplitude
25		Ener-90-50-End	Energy between final 90:50% peak amplitude

26		Ener-50-10-End	Energy between final 50:10% peak amplitude
27		Ener-10-0-End	Energy between final 10:0% peak amplitude
28	Frequency	PulseDomFreq	Dominant frequency of the pulse
29		PulseFundFreq	Fundamental frequency of the pulse
30		PulseMinFreq	Minimum of dominant frequency in the pulse
31		PulseMaxFreq	Maximum of dominant frequency in the pulse
32		PulseOnFreq	Onset pulse dominant frequency
33		PulseOffFreq	Offset pulse dominant frequency
34	Intensity	PulseHalfFM	Prop of duration to reach half frequency Modulation
35		Tuning-6dBSPL	Tuning: peak freq/bandwidth at 50% peak amplitude (Q-20dBSPL)
36		Tuning-6dBSPL	Tuning: peak freq/bandwidth at 10% peak amplitude (Q-20dBSPL)
37		relAmpl-H1	Relative amplitude of harmonic 1
38		relAmpl-H3	Relative amplitude of harmonic 3

5.4 Results

Call samples from three individuals of *A. latidisca* (BH06, BH26 and BH30) were recorded within the study period. These call samples were distinct from the apparent release calls emitted when males were handled, and are considered as advertisement calls.

Generally, the three individuals produced a two-phase call consisting of two distinct segments. Segment A consisted of 3–5 introductory notes (5–12 pulses; mean duration 0.4 seconds each note) separated by inter-note interval longer than then the note and segment B a long, repeated and uninterrupted note (112–155 pulses) up to 10 seconds (Table 5.4).

Table 5.4: Call distinctions in segment A and B pulse trains of the three calling individuals.

Field number	**SVL (mm)**	**T (˚C)**	**Note**		**Note duration (s)**		**Note interval (s)**
			Segment A	Segment B	Segment A	Segment B	
BH06	43.18	18.7	5	1	0.44 ± 0.15	7.8	0.88 ± 0.36
BH26	43.80	17.5	3	1	0.43 ± 0.06	10.3	0.87 ± 0.06
BH30	43.10	20.8	4	1	0.43 ± 0.25	8.2	0.85 ±0.19

There was negative correlations between the snout-vent lengths of the three individuals, fundamental frequencies (r_A = -0.710, N = 3, $p > 0.05$; r_B = -0.370, N = 3, $p > 0.05$) and dominant frequency of segment A and segment B calls (r_A = -0.712, N = 3, $p > 0.05$; r_B = -0.360, N = 3, $p > 0.05$). Note repetition rates were positively correlated with the basal body temperature of calling toads (r = 0.359, N = 3, $p > 0.05$).

Figure 5.1a, b and c show the sonogram and oscillogram of three individual male *A. latidisca* (field number: BH06, BH26 and BH30).

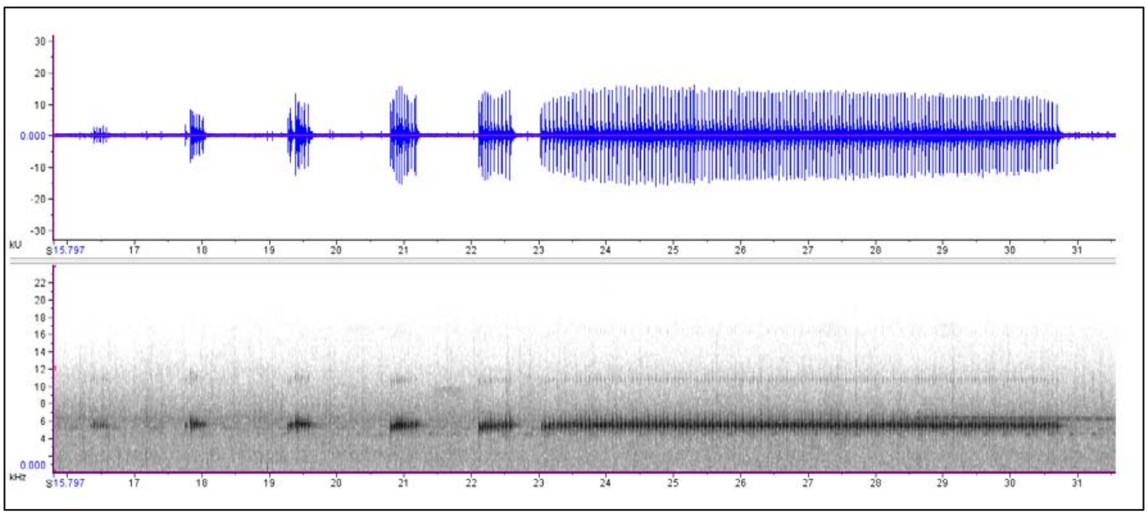


Figure 5.1a: Sonogram and oscillogram of an advertisement call in *Ansonia latidisca* (field number: BH06). The recording was made on 11 February 2012 at 1810 hour.

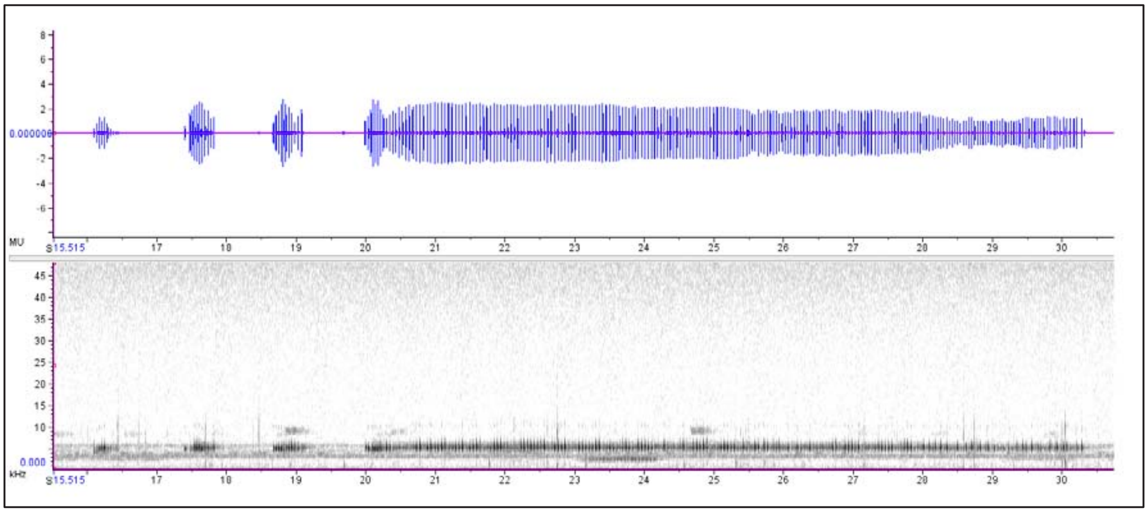


Figure 5.1b: Sonogram and oscillogram of an advertisement call in *Ansonia latidisca* (field number: BH26). The recording was made on 26 August 2012 at 1923 hour.

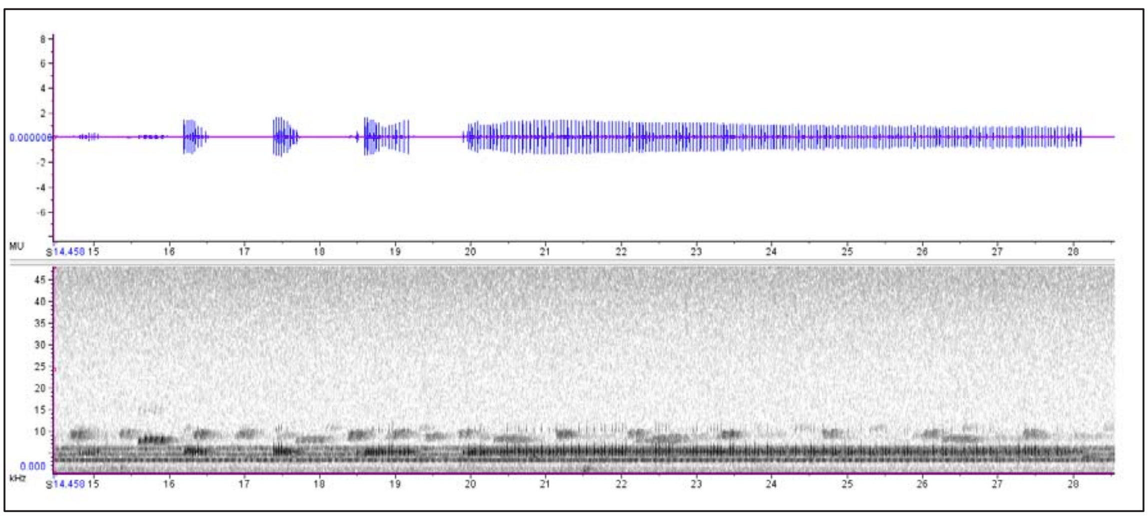


Figure 5.1c: Sonogram and oscillogram of an advertisement call in *Ansonia latidisca* (field number: BH30). The recording was made on 29 September 2012 at 1913 hour.

Table 5.5 tabulates the selected call variables of the three calling individuals. Harmonics are not evident in both segments of calls and energy of the notes spread over the mean range 5.1−5.5 kHz. Mean dominant frequency for segment A and B are around 5.5 kHz, and the fundamental frequency is about 2.7 kHz. The maximum call frequencies for both segment A and B are 5.5 kHz and the minimum call frequencies are around 5.1 kHz.

Table 5.5: Comparison of selected call variables of three individual male *Ansonia latidisca*. * A and B denotes type A and B pulse trains.

Field number	SVL (mm)	T (°C)	Dominant frequency (hz)		Fundamental frequency (hz)		Note repetition rate (note/s)		Maximum frequency (hz)		Minimum frequency (hz)	
			A	B	A	B	A	B	A	B	A	B
BH06	43.18	18.3	5536 ± 175	5517 ± 49	2768 ± 87	2759 ± 24	5	1	5642 ± 186	5614 ± 112	5411 ± 152	5389 ± 89
BH26	43.80	17.2	5319 ± 179	5435 ± 30	2659 ± 89	2717 ± 15	3	1	5378 ± 140	5452 ± 84	4924 ± 224	5046 ± 78
BH30	43.10	21.2	5399 ± 153	5430 ± 50	2699 ± 77	2715 ± 25	4	1	5476 ± 209	5491 ± 141	4830 ± 210	4938 ± 216
Mean			5452 ± 176	5464 ± 59	2730 ± 87	2732 ± 30	4	1	5548 ± 199	5523 ± 134	5123 ± 335	5142 ± 233

Continued from top…

Field number	PulOn_90	PulOn_Peak	PulseOn Freq (hz)	PulseOff Freq (hz)	Ener-10-50-Beg	Ener-0-10 Beg	Ener-90-50-End	Ener-50-90-Beg	PulseHalf FM	
									A	B
BH06	3.98 ± 1.39	3.98 ± 1.39	5537 ± 137	5513 ± 134	0	0.04 ± 0.02	0.03 ± 0.002	0.10 ± 0.05	0.22	0.13
BH26	1.26 ± 0.25	1.26 ± 0.25	5241 ± 188	5034 ± 107	0	0	0.03 ± 0.01	0.01 ± 0	0.05	0.24
BH30	0.85 ± 0.11	0.85 ± 0.11	5443 ± 110	5012 ± 225	0	0	0.01 ± 0	0	0.27	0.13
Mean	2.03 ± 1.7	2.03 ± 1.7	5407 ± 151	5186 ± 283	0	0	0.02 ± 0.01	0.04	0.18	0.17

5.4.1 PCA Analysis

The results of the PCA performed with 38 call variables showed that the first principal component (PC1) explained 78.9% of the variance and PC2 21.1% of the variance. Only PC1 was retained for interpretation as it has the highest eigenvalues of 29.2. Out of 38 variables tested, 33 variables were found meaningful highlighted with an arterisk in Figure 5.2 (see Appendix 3 for the factor loadings of all the 38 call variables).

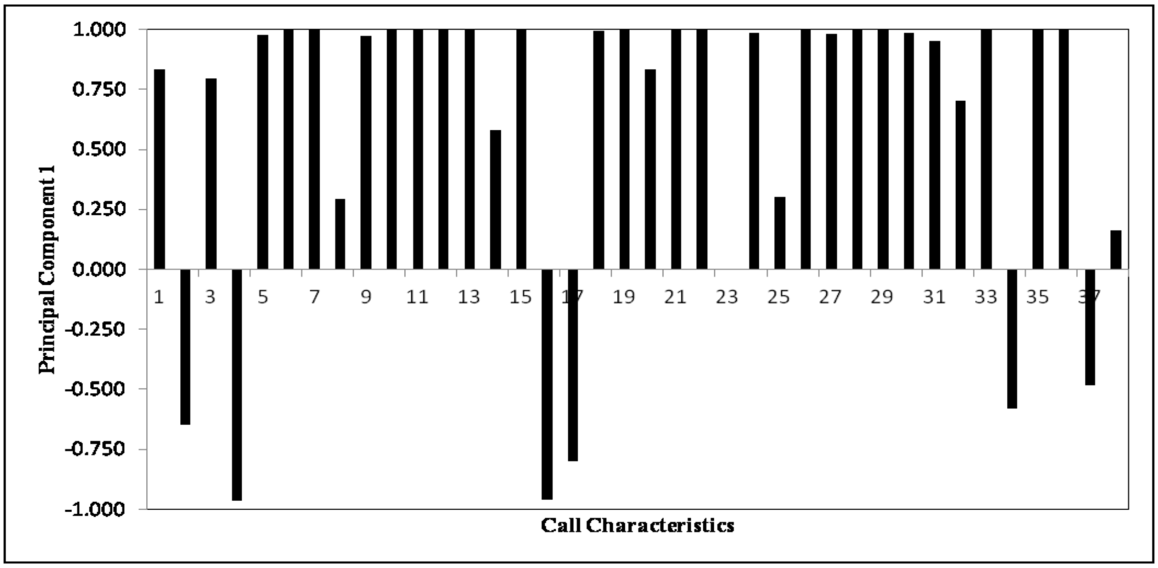


Figure 5.2: Principal Component Analysis 1 (PCA1) of call characteristics in advertisement calls of *A. latidisca* with meaningful variables highlighted with an asterisk:

(1*) Note, (2*) note duration, (3*) note gap, (4*) note repetition rate, (5*) pulse max amplitude relative to call max amplitude, (6*) pulse duration between 0%, (7*) pulse duration between 10%, (8) pulse duration between 50%, (9*) pulse duration between 90% (sustain), (10*) pulse time for onset to 90% (attack), (11*) pulse time for onset to peak (rise), (12*) pulse time for onset to offset (fall), (13*) pulse time for 90% to offset (decay), (14*) pulse shape (10:50% onset/10:90% onset), (15*) pulse shape (10:50% offset/10:90% offset), (16*) pulse interval, (17*) pulse period (time peak to peak), (18*) pulse duration/period (duty cycle), (19*) crest factor, (20*) energy between initial 0:10% peak amplitude, (21*) energy between initial 10:50% peak amplitude, (22*) energy between initial 50:90% peak amplitude, (23) energy between initial 90%: peak amplitude, (24*) energy between final peak: 90% peak amplitude, (25) energy between final 90:50% peak amplitude, (26*) energy between final 50:10% peak amplitude, (27*) energy between final 10:0% peak amplitude, (28*) dominant frequency of the pulse, (29*) fundamental frequency of the pulse, (30*) minimum of dominant frequency in the pulse, (31*) maximum of dominant frequency in the pulse, (32*) onset pulse dominant frequency, (33*) offset pulse dominant frequency, (34) prop of duration to reach half frequency modulation, (35*) tuning: peak freq/bandwidth at 50% peak amplitude (Q-6dBSPL), (36*) tuning: peak freq/bandwidth at 10% peak amplitude (Q-20dBSPL), (37) relative amplitude of harmonic 1, (38) relative amplitude of harmonic 3.

5.5 Discussion

Calls of *A. latidisca* could be heard as early as 1700 hours, after or during light precipitation, but most calling individuals went quiet upon searches probably due to disturbance to the vegetation, resulting in low number of recordings made during the study period. In addition, calls were hardly heard during the beginning until the peak dry period of 2012. The first individual was recorded on 11 February at 1810 hour, calling from a tree trunk (wet seasons; January–March and November–December) and the subsequent two individuals were caught on 26 August, at 1923 hour and 29 September, at 1913 hour, respectively, calling on a rock at a water seepage (presumably the end of the dry season).

The advertisement calls of *A. latidisca* are divided into two segments; A and, B following A. The introductory call (A) or the shorter call consists of 5–12 pulses, followed by a longer, repeated and uninterrupted note (112–155 pulses, segment B) up to over 10 seconds. While most calls of Bornean *Ansonia* species comprises relatively long trills composed of repeated note series (refer to Table 5.2); only *A. spinulifer* has two call variables most similar to *A. latidisca*; a short and a long call. Call characteristics comparison within the same genus was not viable as there was no raw data.

Negative correlations between call energies against body size suggest smaller males produced relatively lower frequency calls in response to the larger males. Males can use the frequencies of other males' calls to assess their body size (Wells, 2007), and could possibly lead to deceptive information by making a male appear larger (Bee at al., 2000). On the other hand, positive correlation between note repetition rate (Bee et al., 2000) and basal body temperature demonstrates temperature constraints on the contractile properties of the trunk and laryngeal muscles involved in the production of the call (Valetti and Martino, 2012).

Results from PCA show four groups of call variables, most notably call notes, pulse durations, call energies and call frequencies, to be meaningful, and to discriminate groups conspecifically. This result is consistent with Zainudin et al. (2010), who noted three of five variable groups such as pulse duration, call energy and call frequency, dominating the highest factor loadings, best to describe the calls.

5.6 Conclusions

The bioacoustics of male *A. latidisca* can be divided into two segments, a short call and a long call following the former. The short call is of 5–12 pulses (duration = 0.4 seconds) and followed by a long, repeated and uninterrupted note (112–155 pulses) up to 10 seconds. Mean dominant frequency for segment A and B are around 5.5 kHz, and the fundamental frequency is about 2.7 kHz. While frequency modulation was not evident, results from PCA analysis showed four different call variable groups were useful, notably call notes, pulse durations, call energies and call frequencies.

Negative correlations between call energies against body size suggest smaller males to produce relatively lower frequency calls in response to the larger males, positive correlation between note repetition rate and basal body temperature demonstrates that temperature can affect the auditory threshold of frogs.

CHAPTER 6

CONCLUSIONS

Dubbed as one of the world's top 10 most wanted lost frogs in a campaign by Conservation International and International Union for Conservation of Nature and Natural Resources (IUCN) Amphibian Specialist Group, in 2011, *Ansonia latidisca*, a montane species, survives on the steep mountain ridges of upper Gunung Penrissen, in western Sarawak, in Malaysian Borneo.

Results from stomach flushing 46 adults *A. latidisca* reveal 12 categories of food, dominated by formicids. The dietary profile fits the description of an ant specialist, and the species thus has an active preference for feeding on small prey showing clumped distribution.

In addition, the discovery of *A. latidisca* as the first arboreal species within the genus provides insights into its poorly understood lifestyle. The first attempt to study its orientation was made, when activities over a 24 hour period was tracked. During this brief study, the single individual investigated did not leave arboreal habitats.

The calls of three male *A. latidisca* can be divided into two segments, a short call and a long one, following the former. The introductory call or the shorter call consists of 5–12 pulses (duration = 0.4 seconds), followed by a longer, repeated and uninterrupted note (112–155 pulses, segment B), up to over 10 seconds.

Finally, these studies have added important baseline knowledge to a relatively poorly known Bornean endemic. While the brightly coloured skin, that show patches of red, in the species can be used as a warning to predators (aposematic colouration), it can also be considered to show surface mimicry in providing camouflage, mimicking tree trunks that are festooned with lichens and mosses at the elevation where the species occurs. Additional specializations in morphology noted in the study species include dilated fingers tips and relatively elongated body and limbs, features not observed in congeneric species.

6.1 Recommendations

An attempt to identify ants up to the level of genus in future studies would add value to the dietary data. By looking at the predominant ant genera being consumed by the toads, and determing what proportion are noxious, one can understand the relative contribution of each prey type to the alkaloid sequestration for presumed chemical defense in the toad.

An more extensive home range study need to be conducted, perhaps using radio-telemetry, or the improvised, sewing machine bobbin as used in this study to further understand the movement of the species. Reliable home range data are essential for developing conservation management plans for the species.

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Appendix 1: Collection data on *Ansonia latidisca* from the present study.

Field number	M/Day	SVL (mm)	Weight(g)	Sex	Tag colour	Remark
BH01	13.1.2012	41.50	3.2	M	Blue	
BH03	10.2.2012	43.74	3.8	M	Red	
BH04	10.2.2012	37.78	4.2	M	Green	
BH01 (Recaptured)	10.2.2012	40.77	3.3	M	Blue	
BH06, OJJ0051	11.2.2012	43.18	4.6	M	…	OJJ0051, preserved
BH07	11.2.2012	37.12	3.7	M	Pink	
BH08	11.2.2012	40.35	3.4	M	Yellow	
BH08 (Recaptured)	24.2.2012	40.83	3.5	M	Yellow	
BH03 (Recaptured)	24.2.2012	41.16	3.8	M	Red	
BH15	6.4.2012	44.86	3.7	M	Yellow	
BH16	4.5.2012	40.30	4.1	M	Double red	
BH16 (Recaptured)	18.5.2012	41.70	3.2	M	Double red	
BH18	15.6.2012	43.44	3.4	M	Yellow and green	
BH19	29.6.2012	41.48	4.6	M	Pink and green	
BH21	30.6.2012	44.46	3.8	M	…	Record lost
BH03 (Recaptured)	28.7.2012	40.76	3.6	M	Red	
BH26	26.8.2012	43.80	4.4	M	…	OJJ105, preserved
BH27	26.8.2012	41.58	5.7	M	…	Record lost
BH30	29.9.2012	43.10	4.4	M	…	OJJ110, preserved
BH31	26.10.2012	41.38	3.7	M	…	Record lost
BH32	26.10.2012	41.52	3.7	M	…	Record lost
BH33	26.10.2012	42.70	4.2	M	…	Record lost
BH34	27.10.2012	41.92	3.5	M	…	Record lost
BH35	27.10.2012	41.90	3.9	M	…	Record lost
BH19 (Recaptured)	27.10.2012	42.10	4.0	M	Pink and green	
BH36	23.11.2012	41.70	3.6	M	Double blue	
BH37	23.11.2012	42.86	3.6	M	Double yellow	
BH38	14.12.2012	40.98	3.7	M		OJJJ133, preserved
BH39	14.12.2012	40.48	3.6	M	Double green	
BH40	14.12.2012	43.10	3.9	M	Double pink	

	M/Day	SVL (mm)	Weight (g)	Sex	Tag Colour	Remarks
BH05	10.2.2012	50.37	4.2	F	…	Record lost
BH09	12.2.2012	48.00	4.6	F	…	Record lost
BH10	12.2.2012	50.88	5.1	F	…	Record lost
BH11	25.2.2012	50.87	5.1	F	…	Record lost
BH12	25.2.2012	50.74	5.9	F	Caught back for captive breeding	-
BH13	25.2.2012	52.87	10.5	F	Red	-
BH14	9.3.2012	42.76	4.0	F	Green	-
BH17	19.5.2012	51.32	6.9	F	Blue	-
BH20	30.6.2012	53.44	10.1	F	Yellow and red	-
BH20 (Recaptured)	28.7.2012	56.28	12.7	F	Yellow and red	-
BH22	28.7.2012	47.70	6.6	F	Double red	
BH23	28.7.2012	52.66	9.4	F	Red and pink	
BH24	24.8.2012	54.00	11.0	F	Red and blue	
BH25	24.8.2012	51.20	5.8	F	Yellow and green	
BH20 (Recaptured)	26.8.2012	55.50	12.6	F	Yellow and red	

Appendix 2: Field data on microhabitat use by *Ansonia latidisca*.

Field number	Sex	Date	SVL (mm)	Horizontal position	Vertical position	Substrate type
BH01	M	13.1.2012	41.50	2	2	2
BH02	F	10.2.2012	53.35	2	2	2
BH03	M	10.2.2012	43.74	2	2	2
BH04	M	10.2.2012	37.78	2	2	2
BH05	F	10.2.2012	50.37	2	2	3
BH01	M	10.2.2012	40.77	2	2	2
BH06	M	11.2.2012	43.18	2	2	3
BH07	M	11.2.2012	37.12	2	2	2
BH08	M	11.2.2012	40.35	2	2	2
BH09	F	12.2.2012	48.00	2	2	3
BH10	F	12.2.2012	50.88	2	2	3
BH08**	M	24.2.2012	40.83	2	2	3
BH03**	M	24.2.2012	41.16	2	2	2
BH11	F	25.2.2012	50.87	2	2	3
BH12	F	25.2.2012	50.74	2	2	3
BH13	F	25.2.2012	52.87	2	2	3
BH14	F	9.3.2012	42.76	2	2	3
BH15	M	6.4.2012	44.86	2	2	2
BH16	M	4.5.2012	40.30	2	2	2
BH16**	M	18.5.2012	41.70	2	2	3
BH17	F	19.5.2012	51.32	2	2	2
BH18	M	15.6.2012	43.44	2	2	2
BH19	M	29.6.2012	41.48	2	2	3
BH21	M	30.6.2012	44.46	2	2	3

Under the category "horizontal position", "1" refers to 0−1 m and "2" denotes > 1 m, to nearest water source. For the category "vertical position", "1" refers to on ground and "2" denotes above ground and as for the category "substrate type", "1" refers to rock, "2" as leaf and "3" as tree trunk.

Field number	Sex	Date	SVL (mm)	Horizontal position	Vertical position	Substrate type
BH20	F	30.6.2012	53.44	2	2	3
BH03**	M	28.7.2012	40.76	2	2	3
BH20**	F	28.7.2012	56.28	2	2	3
BH22	F	28.7.2012	47.70	2	2	3
BH23	F	28.7.2012	52.66	2	2	3
BH24	F	24.8.2012	54.00	2	2	3
BH25	F	24.8.2012	51.20	2	2	2
BH20**	F	26.8.2012	55.50	2	2	3
BH26	M	26.8.2012	43.80	1	2	1
BH27	M	26.8.2012	41.58	2	2	3
BH30	M	29.9.2012	43.10	1	2	1
BH31	M	26.10.2012	41.38	1	2	2
BH32	M	26.10.2012	41.52	1	1	1
BH33	M	26.10.2012	42.70	1	1	1
BH34	M	27.10.2012	41.92	1	2	1
BH35	M	27.10.2012	41.90	1	2	2
BH19**	M	27.10.2012	42.10	2	2	3
BH36	M	23.11.2012	41.70	2	2	3
BH37	M	23.11.2012	42.86	1	2	2
BH38	M	14.12.2012	40.98	1	2	2
BH39	M	14.12.2012	40.48	1	2	2
BH40	M	14.12.2012	43.10	1	2	1

Under the category "horizontal position", "1" refers to 0−1 m and "2" denotes > 1 m, to nearest water source. For the category "vertical position", "1" refers to on ground and "2" denotes above ground and as for the category "substrate type", "1" refers to rock, "2" as leaf and "3" as tree trunk.

Appendix 3: Factor loadings of all 38 call variables.

Call variables	Factor loading
Note	0.833
Note duration	-0.648
Note interval	0.796
Note repetition rate	-0.964
RelPulsePeak	0.977
PulDur_0	0.999
PulDur_10	0.977
PulDur_50	0.293
PulDur_90	0.973
PulOn_90	0.998
PulOn_peak	0.998
PulOff_peak	1
PulOff_90	0.999
PulShapeOn	0.58
PulShapeOff	-.997
PulInter	-0.96
PulPeriod	-0.798
PulDuty	0.996
CrestFactor	0.998
Ener_0-10_Beg	0.833
Ener_10-50_Beg	0.999
Ener_50-90_Beg	1
Ener_90-Peak_Beg	0
Ener_Peak-90_End	0.984
Ener_90-50_End	0.301
Ener_50-10_End	1
Ener_10-0_End	0.981
PulseDom#Freq	0.997
PulseFundFreq	0.997
PulseMinFreq	0.985
PulseMaxFreq	0.954
PulseOnFreq	0.702
PulseOffFreq	1
PulseHalfFM	-0.579
Tuning-6dBSPL	0.999
Tuning-20dBSPL	0.999
relAmpl_H1	-0.485
relAmpl_H3	0.162