AMPHIBIAN MONITORING IN KAKAMEGA FOREST, KENYA

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KEY WORDS

Amphibians, bias, declines, estimators, habitat, Kakamega, monitoring, rainfall, species richness, transect

ABSTRACT

Since the late 1970 there has been increased concern of amphibian decline and extinction. Several causes for the worldwide declines have been suggested and include ultraviolet radiation, predation, pollution, climate change, diseases and habitat modification. To counter this, more research on the subject has been encouraged of which long term monitoring has been suggested as a research method. The study was conducted in Kakamega Forest in Kenya, which is the country's remnant of the once vast Guineo-Congolian forest. A rectangular transect whose sides measured 600 m in total was established and transect walks were carried out every two weeks for two consecutive days between 2002 and 2006. 24 species were targeted in the study and were sampled through VES and AES and data recorded in a GPS and later downloaded. In this study I examined the influence of rainfall, temperature, habitat and moon phases on the activity of frogs in Kakamega Forest. I also determined under which weather conditions sampling was more efficient. When monitoring was carried out by two observers I tested whether their data were similar. Data were analysed using non-parametric methods (Kruskal-wallis and Tukey test), species abundances analysed using EstimateS..Out of the 24 targeted species only 14 were recorded, with a total of 535 specimens being counted mostly at night. Most frogs in Kakamega

Forest were more active in temperatures between 20 and 25^{o}C. There was not much variation and there was no frog activity when the temperature was extremely high. There was rainfall throughout the year and there was no significant differences in the number of frogs counted in rainfall above 200 mm or below 200 mm. There was no significant difference in the number of specimens found in the different vegetation segments in the forest. More amphibians were caught under cloudy, rainy and clear conditions at night than under any weather condition during the day. During the day, more amphibians were caught during cloudy conditions than when it rained or when there was no cloud cover. There was no difference in catch among night conditions and there was no difference between clear and rainy days In Kakamega Forest, night is the best time to sample amphibians. In terms of weather it is best to sample when it is cloudy both during the day and at night. There were no differences in sampling abilities between two observers tested under similar weather conditions.

DECLARATION

I declare that *Amphibian monitoring in Kakamega Forest, Kenya* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Vincent M. Wairimu September 2007

Signed: _____

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I would like to dedicate my work to my wife Regina Wambui and my mother Pauline Wairimu for their love and support.

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Chapter 1: General Introduction

1.1 Amphibian declines

Alford & Richards (1999) noted an increased concern since the late 1970s about amphibian declines. There were more reports during the 1989 First Herpetological Congress in Canterbury, United Kingdom (Barinaga, 1990, Stuart *et al*., 2004). Several causes for the worldwide declines have been suggested and include ultraviolet radiation, predation, pollution, climate change, diseases and habitat modification (Alford & Richards, 1999; Collins & Storfer, 2003). Amphibians are greatly affected because of their low vagility and their breeding requirements as compared to other vertebrates (Mazerolle, 2003).

In September 2005, at a summit convened by the IUCN/SSC Global Amphibian Specialist Group (GASG) in Washington D.C., the "Amphibian Conservation Action Plan" (ACAP) declaration was unveiled (GAA website, 2007). The declaration had four key strategies for amphibian conservation which are:

1. Increased research to boost knowledge on the causes of amphibian declines and extinctions.
2. Ongoing update and documentation of amphibian diversity and their changes in Global Amphibian Assessment (GAA).
3. To develop and implement long term amphibian conservation programs.
4. Rapid (emergency) response to crises.

Many advocates of amphibian decline agree that habitat modification has a tremendous effect on amphibian populations (Collins & Storfer, 2003; Mazerolle, 2003; Murray &

Hose, 2005). This may not be immediately discernible as the effects leading to declines may be gradual. Long term monitoring of amphibians to determine whether the amphibian populations are declining or the frogs' activity varies due to different factors such as predation, pollution, climate change, diseases and habitat modification are needed (Collins & Storfer, 2003; Mazerolle, 2003; Murray & Hose, 2005, Ryan *et al*., 2002). Long term monitoring is one of the strategies in the ACAP declaration.

1.2 Monitoring

Different methods of monitoring amphibian populations have been suggested by Heyer *et al*. (1994). These include species inventory, quadrat sampling, transect sampling, patch sampling, pitfalls with or without drift fences, visual encounter surveys (VES), surveys at breeding sites, acoustic surveys (AS), pitfalls at breeding sites and sampling of amphibian larvae. Application of the different methods will depends on the type of information that one needs to obtain, time available, number of personnel and costs involved. Species inventory determines the species richness in a study area. VES, AS, pitfalls, and surveys at breeding sites are used to provide species relative abundance whereas sampling of amphibian larvae yields both relative abundance and density. Transect, quadrat and patch sampling provide species density (Heyer *et al*., 1994; Rödel & Ernst, 2004; Veith *et al*., 2004).

Transect walks allow for sampling of amphibians on different gradients of habitats and altitudes. This is because amphibians respond differently to different environmental factors (Heyer *et al*., 1994). Additionally, transect walks also help study different individuals within a metapopulation (Alford & Richards, 1999).

Amphibian autoecology is poorly known as many ecological and population biology studies of amphibians concentrate on the reproduction sites. Moreover, in order to

understand the concept of amphibian declines there is a need to study amphibians in a community as compared to studying them individually (Alford & Richards, 1999).

1.3 Study site

Kakamega forest is located in the Western Province of Kenya. It is the country's only remnant of the once vast Guineo-Congolian forest (Fashing & Gathua, 2004; KIFCON, 1994; Köhler *et al*., 2003) exhibiting similar herpetofauna characteristics with that of Central Africa (Köhler *et al*., 2003).

There are over 200 known amphibian species from East Africa (Channing & Howell, 2006) with Kenya having a total of 96 species. For the Kenyan species, 14 are endemics and 6 threatened with extinction (IUCN *et al.*, 2006). Kakamega Forest is the home to 25 known frog species occupying different niches of the forest (Lötters *et al*., 2006). All of the species are categorized as least concern (LC) (IUCN *et al.*, 2006) except *Leptopelis mackayi* and *Xenopus victorianus* which have not been categorised. *L. mackayi* is a new species (Köhler *et al*., 2006). Most frog species in the forest are terrestrial or arboreal, nocturnal and reproducing seasonally in water (Veith, 2004).

1.4 Project sponsorship

This study was carried out as part of the Biodiversity and Global change (BIOLOG) in the German Federal Ministry of Education and Research and undertaken through Biodiversity Transect Analysis in Africa (BIOTA) East Africa. BIOTA EAST comprised of many subprojects and this study falls under subproject E-08: Biodiversity-Change in Frogs from Eastern Africa: Global, Regional or Local Causes? See http://www.biota-africa.org/800/biota_east/subprojects/structure_east_abs1.htm

The study aims

1. To investigate the Kakamega Forest amphibian community activity in relation to temperature, rainfall and habitat along the transect.
2. To determine the number of transect walks required to get representative data of various population estimators and the influence of different abiotic factors on anuran activity.
3. To determine whether there is observer bias when two people undertake transect walks under similar weather conditions.

Chapter 2: Describing the Kakamega Forest amphibian community through transect walks

2.1 Introduction

One important goal when studying a population is to know the number of individuals in a given area. Organisms require different census methods due to their various life histories and ecology (Sutherland, 1996). In most cases, it is not possible to carry out direct counts on every specimen in a given area, hence the need to use samples representing the whole population (Chao, 2005; Heyer *et al*., 1994; Krebs, 1998; Sutherland, 1996).

Various methods are employed in the census of plants and animals (Davies, 2002; Gilbertson *et al*., 1985; Heyer *et al*., 1994; Krebbs, 1998; Sutherland, 1996; White & Edwards, 2000). Their application will depend on the goals of the study, species in question, time available for the study and the cost of undertaking the study (Krebs, 1998; Sutherland, 1996).

2.1.1 Transect walks as a monitoring method

Transect sampling is one of the methods employed in estimating population size and involves sampling along a line or a narrow band (Davies, 2002; Gilbertson *et al*., 1985; Heyer *et al*., 1994; Krebbs, 1998; Sutherland, 1996; White & Edwards, 2000). Transects are either point or linear and can be used for sampling across different vegetation covers. In point transects the observer(s) stand at one point and make observations along the line or band whereas in the case of line transects, the observer(s) move along the line or band (Greenwood, 1996). They can be used either during the day or night (Jaeger, 1994). In this study I used a line transect and the word transects will be referring to the same.

2.1.2 Transects in amphibian monitoring

While large animals e.g. antelopes and elephants have features like dung and footprints that make their detection easy, amphibians are different (Howell, 1996; White & Edwards, 2000). Amphibians are small and their reproductive biology is moisture dependent (Duellman & Trueb, 1986). They occupy different niches in different habitats; they can be arboreal, terrestrial, fully aquatic or living in more than one of the habitats. Their breeding is water-dependent and can be either explosive or prolonged throughout the year (Heyer, *et al*., 1994). Some species are nocturnal with a few being diurnal. Methods applied in their monitoring should therefore account for both day and night (Heyer *et al*., 1994; Veith *et al*., 2004).

The advantage of sampling across different habitat gradients on the same transect makes transect sampling a good method for use in tropical rain forests. Different habitats may harbour different amphibians and it is therefore prudent to employ a method that accounts for each vegetation type. Transect walks in amphibian monitoring apply both visual and acoustic surveys. Acoustic encounter surveys (AES) involve the use of male advertisement calls and are good for cryptic species but are gender biased (Rödel & Ernst, 2004; Veith, *et al.*, 2004). On the other hand visual encounter surveys (VES) are not gender biased but have reduced incidence of detection. The above methods can be standardised in space, time and number of observers to be applied in transects and are referred to as standardised visual transect surveys (SVTS) and standardised acoustic transect surveys (SATS) (Jaeger, 1994; Rödel & Ernst, 2004; Veith, *et al*., 2004).

Transect sampling can be combined with quadrat sampling to give a rectangular transect. This is useful in studies that deal with community composition, species richness, relative abundance, intra-and-inter species comparisons and species densities in relation to space and time.

One of the ACAP strategies is to increase the knowledge of the causes of amphibian declines. A particular area where more research is needed is the connection between climate change and amphibian declines. Alford & Richards (1999) noted that understanding the problem of

amphibian declines necessitates understanding species in relation to a metapopulation's ecology. Amphibians also interact differently to some abiotic factors like humidity, precipitation, temperature and moon phases (Bertoluci & Roudrigues, 2002; Blair, 1960, 1961; Bowker & Bowker, 1979; Saenz, *et al.*, 2006; Stewart, 1985, 1995). The influence of these factors on amphibians has not been well studied in wet tropical forests (Crump, 1994). Studies in this area will add knowledge to the little known African amphibian diversity (Poynton, 1996).

2.1.3 Kakamega Forest amphibian community

Understanding the life history of amphibians helps in deciding which method of sampling to use (Heyer *et al.*, 1994). This makes locating of specimens easier as the observer has a clue about where to find the specimens and when they are likely to be found. It also helps in tracking changes in species activity patterns, which may indicate declines and extinctions.

By the time the study was being conducted 24 species of frogs were known from Kakamega Forest and were targeted for monitoring. Schick *et al.*, (2005), put the number as 25 species of frogs. Currently, according to National Museums of Kenya (NMK) database there are 101 species of frogs in Kenya, 26 of which are known from Kakamega Forest. The species monitored, their ecology and life history are described below and summarised in Table 2.1.

2.1.3.1 Family Arthroleptidae

Leptopelis bocagii (Günther, 1865)

This is a ground dwelling tree frog found in the savanna. Males call from the ground, in burrows or even up to two metres above the ground on trees. Eggs are laid deep in the ground during rains. The biology of this species' tadpole is not known (Channing & Howell, 2006; Schiøtz, 1999).

Leptopelis mackayi Köhler, Bwong, Schick, Veith, and Lötters, 2006

This is an arboreal frog currently known only from Kakamega Forest, both in primary and secondary forest. The species is currently threatened by logging undertaken in this forest. The species is a prolonged breeder with breeding taking place between April and September. Eggs are thought to be deposited in the soil with the hatching tadpoles moving into shallow waters (Köhler *et al*., 2006).

2.1.3.2 Family Bufonidae

Amietophrynus maculatus (Hallowell, 1854)

This species is found in all habitat types ranging from degraded forests to moist savanna. *Amietophrynus maculatus* is an explosive breeder. The males call concealed under vegetation and the eggs are laid in strings. The tadpoles are lentic (Channing & Howell, 2006; Rödel & Agyei, 2003).

Amietophrynus kisoloensis (Loveridge, 1932)

This frog is associated with rivers and cool moist highland forests with males calling from shallow streams beneath vegetation. (Channing & Howell, 2006).

2.1.3.3 Family Hyperoliidae

Afrixalus osorioi (Ferreira, 1906)

This is a forest species found within bushland, rainforest and grasslands. Its reproductive biology is unknown (Lötters *et al*., 2006; Schiøtz, 1999).

Afrixalus quadrivittatus (Werner, 1908)

This forest species is found in savanna and grasslands. Eggs are deposited in small clamps between leaves in or above water. The tadpoles are not known (Channing & Howell, 2006; Schiøtz, 1999).

Hyperolius acuticeps Ahl, 1931

This nocturnal arboreal species is found in swamps and ponds in disturbed primary forest (Lötters *et al*., 2004). Males are found calling in reeds and vegetation next to deep water and can call up to three metres above the ground (Channing & Howell, 2006; Lötters *et al*., 2004). Eggs are deposited in water individually or in clusters (Channing & Howell, 2006; Lötters *et al*., 2004). It is intermediate between an explosive and prolonged breeder with tadpoles being lentic and omnivorous (Channing & Howell, 2006; Lötters *et al*., 2004).

Hyperolius cinnamomeoventris Bocage, 1866

This is an arboreal nocturnal reed frog found in primary forest and bushes similar to *H. lateralis* (Lötters *et al*., 2004; Schiøtz, 1975, 1999). The frog is found in swampy areas and ponds and is an intermediate between explosive and prolonged breeder (Lötters *et al*., 2004; Vonesh, 2000). Eggs are laid attached to vegetation above water and hatch after 15 days with tadpoles being lentic and omnivorous (Channing & Howell, 2006; Lötters *et al*., 2004; Vonesh, 2000).

Hyperolius kivuensis Ahl, 1931

The nocturnal and arboreal *H. kivuensis* is found in disturbed primary forest and its edges, in swamps or ponds. Males call up to two metres above the ground with females found migrating inside the forest (Channing, 2001; Lötters *et al*., 2004).

They are prolonged breeders with their breeding season between March and October with eggs being laid in clutches on vegetation (Lötters *et al*., 2004; Vonesh, 2000). The eggs hatch after

nine days (Channing & Howell, 2006) with their tadpoles being lentic and omnivorous (Lötters *et al*., 2004).

Hyperolius lateralis Laurent, 1940

The nocturnal and arboreal *Hyperolius lateralis* is found living in disturbed primary forest and its edges (Lötters *et al*., 2004, 2006). It breeds during the wetter months (Lötters *et al*., 2004; Vonesh 2000), with males calling from vegetation about 1.5 m above the ground (Lötters *et al*., 2004). This species is intermediate between an explosive and prolonged breeder with eggs being laid in clutches on vegetation above the water surface (Lötters *et al*., 2004; Vonesh, 2000). Tadpoles develop after 14 days which are lentic and omnivorous (Channing & Howell, 2006; Lötters *et al*., 2004).

Hyperolius viridiflavus (Duméril and Bibron, 1841)

This is a complex *Hyperolius* group made up of frogs varying in colour but similar in life history, evolution, biology and morphology (Schiøtz, 1999; Wieczorek *et al*., 2000). In Kakamega Forest, they have been found in disturbed primary forest and ponds. They are prolonged breeders with females observed migrating in the forest. Their tadpoles are found in still or slow flowing waters and are omnivorous (Lötters *et al*., 2004).

Kassina senegalensis (Duméril and Bibron, 1841)

The bubbling kassina is common throughout sub-Saharan Africa. The species breeds in April and May in East Africa in shallow waters. The eggs sink into the water, and the tadpoles are omnivorous (Razzetti & Msuya, 2002; Rödel & Ernst, 2001).

2.1.3.4 Family Dicroglossidae

Hoplobatrachus occipitalis (Günther, 1858)

The cannibalistic giant swamp frog is found in swamps, deep permanent ponds, lakes and rivers. It is an all year round breeder, producing up to 3752 eggs per clutch, which attaches individually on the pond bottom. The tadpoles are carnivorous (Rödel, 2000).

2.1.3.5 Family Phrynobatrachidae

Phrynobatrachus graueri (Nieden, 1911)

A small frog found in damp litter near rivers. The frog's reproductive biology is not known (Channing & Howell, 2006).

Phrynobatrachus minutus (Boulenger, 1895)

This frog is found in moist grassland, cleared forest, herbaceous vegetation, rocks found at the swampy margins of lakes, rivers, streams and temporary pools. It is active at night although males can be heard calling in daytime (Largen, 2001; Veith *et al*., 2004).

Phrynobatrachus natalensis (Smith, 1849)

This is a widely distributed puddle frog found in open grassland, streams, ponds and puddles and active both during the day and at night (Bowker & Bowker 1979; Channing & Howell, 2006; Largen, 2001; Veith *et al*., 2004). The males call outside water at the edge of ponds and puddles (Crutsinger *et al*., 2001).

2.1.3.6 Family Pipidae

Xenopus victorianus Ahl, 1924

This is a fully aquatic species found in arid areas, savanna and in forests. Its breeding is unknown. The large tadpoles reach up to 80 mm, and are filter feeders. The smaller ones are found around vegetation and the bigger ones in deep water (Tinsley & Kobel, 1996; Channing & Howell, 2006).

2.1.3.7 Family Ptychadenidae

Ptychadena anchietae (Bocage, 1868)

This is a savanna species found in grasses near water. It breeds in puddles with the eggs floating on the surface. The tadpoles are omnivorous (Channing & Howell, 2006).

Ptychadena mascareniensis (Dümeril and Bibron, 1841)

This species is found along streams, and in standing water (Channing & Howell, 2006; Vences *et al*., 2004). Breeding takes place during the short and long rains with tadpoles living in temporary pools. Metamorphosis takes place after nine days (Channing & Howell, 2006).

Ptychadena oxyrhynchus (Smith, 1849)

P. oxyrhynchus is a widely distributed savanna species whose males call from water edges though away from water. Eggs are laid in strings, which break away. The eggs float and develop into grey tadpoles (Channing & Howell, 2006).

Ptychadena porosissima (Steindachner, 1867)

This species is found in moist grasslands and high altitude forests with their males calling under vegetation. Eggs are laid in shallow grassy pools and develop into brown tadpoles (Channing & Howell, 2006).

Ptychadena taenioscelis Laurent, 1954

This is a widely distributed grassland species, whose males call early in the evening. Eggs are laid in shallow water and sink into water. The tadpoles of this species are not known (Channing & Howell, 2006).

2.1.3.8 Family Pyxcephalidae

Amietia angolensis (Bocage, 1866)

This is a terrestrial species found in grasslands, streams and rivers. It is a prolonged breeder, with December being the peak. Eggs are deposited in shallow waters, ponds and flowing waters. Tadpoles lie in sunny parts of the water (Channing & Howell, 2006).

2.1.3.9 Family Ranidae

Hydrophylax cf. albolabris (Hallowell, 1856)

A forest species that inhabits disturbed primary forests and breeds in puddles. Large quantities of eggs are found floating on the surface. The tadpoles live in flowing water (Channing & Howell, 2006; Rödel & Agyei, 2003).

Table 2.1. Summary of the species expected in Kakamega forest and the habitats they occupy

Species name	Habitat
Leptopelis bocagii (Günther, 1865)	A/T
Leptopelis mackayi Köhler, Bwong, Schick, Veith, and Lötters, 2006	A
Amietophrynus maculatus (Hallowell, 1854)	T
Amietophrynus kisoloensis (Loveridge, 1932)	A
Afrixalus osorioi (Ferreira, 1906)	A
Afrixalus quadrivittatus (Werner, 1908)	A
Hyperolius acuticeps Ahl, 1931	A
Hyperolius cinnamomeoventris Bocage, 1866	A
Hyperolius kivuensis Ahl, 1931	A
Hyperolius lateralis Laurent , 1940	A
Hyperolius viridiflavus (Duméril and Bibron, 1841)	A
Kassina senegalensis (Duméril and Bibron, 1841)	A/T
Hoplobatrachus occipitalis (Günther, 1858)	T/Q
Phrynobatrachus graueri (Nieden, 1911)	T
Phrynobatrachus minutus (Boulenger 1895)	T
Phrynobatrachus natalensis (Smith, 1849)	T
Xenopus victorianus Ahl, 1924	Q
Ptychadena anchietae (Bocage, 1868)	T
Ptychadena mascareniensis (Dumeril & Bibron, 1841)	T
Ptychadena oxyrhynchus (Smith, 1849)	T
Ptychadena porosissima (Steindachner, 1867)	T
Ptychadena taenioscelis Laurent, 1954	T
Amietia angolensis (Bocage, 1866)	T
Hydrophylax cf. albolabris (Hallowell, 1856)	T

Legend

A = arboreal, T= terrestrial and Q= fully aquatic

2.2 Aims

The goal in this chapter is to determine which species can be found and where they can be found along the transect. I also sought to know when these frogs are active and the influence of rainfall, temperature and seasons on their activity.

2.3 Materials and methods

2.3.1 Study site

Kakamega Forest

Kakamega Forest (figure 2.1) is located in Kakamega district in the Western Province of Kenya. The location of the forest is 40 km NW of Lake Victoria with an altitude of 1 500 to 1 700 m above sea level. The forest is the only Kenyan remnant of the once vast Guineo-Congolean rainforest (Blackett, 1994; Fashing & Gathua, 2004) and lies between 34^{o} 37' 5" to 35^{o} 9' 25" E and 0^{o} 32' 24" to 0^{o} 2' 52" S (Lung & Schaab, 2004).

According to data from the Kenya Meteorological Service, Malava station which is near the forest, the area received an annual rainfall of between 1 097 and 2 231 mm from 1996 to 2006 with the highest amounts of rainfall in April and May. It is a highland rainforest type, which has two major rainfall seasons. The heavy rain season is between March and July while the short rains fall between September and November. There is a dry period between December and February (Blackett 1994; Fashing & Gathua, 2004; KIFCON 1994). The forest has a moderately warm and humid climate with temperatures oscillating between 15 and 27 ^{o}C. (Blackett, 1994; KIFCON, 1994).

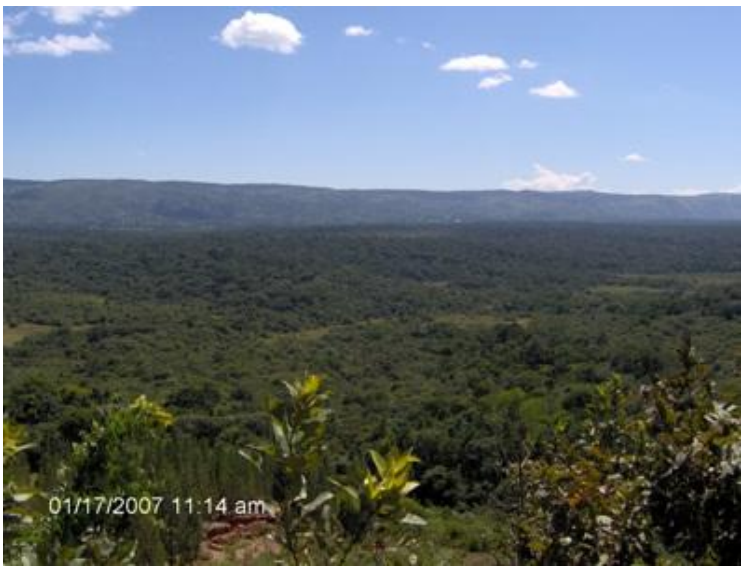


Figure 2.1. Patchwork of vegetation in Kakamega Forest with the Nandi hills in the background

Kakamega Forest covers an area of 240 km^2 of which only 100 km^2 is occupied by fragmented indigenous forest (Fashing & Gathua, 2004; KIFCON, 1994). The forest borders North and South Nandi Forests and is surrounded by small forest fragments which may have been connected in former times (Figure 2.2).

Kakamega Forest was first gazetted as a trust forest in 1933. Yala and Isecheno Forest fragments were incorporated into the forest reserve in 1967. In 1986, Kisere Forest fragment was added to Kakamega Forest and gazetted as a forest reserve under the management of the Kenya Wildlife Service (KWS) (Bennun & Njoroge, 1999).

The forest has been under pressure from the increase in the human population surrounding it. The main sources of pressure are tree logging, fuel wood collection, charcoal burning, farming; pit sawing, forest fires and gold prospecting (Blackett, 1994; Mitchell, 2004).

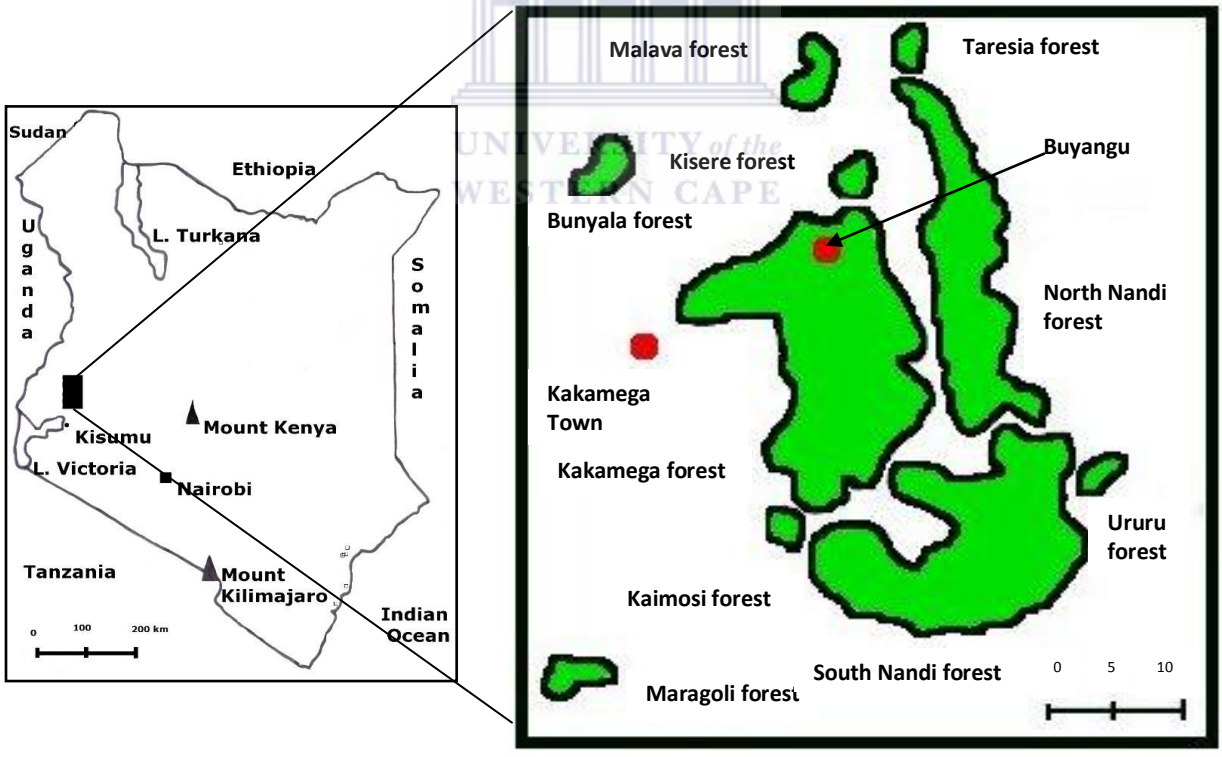


Figure 2.2. Location of Kakamega Forest and the associated forest fragments (after Lung & Schaab, 2004).

Despite this, Kakamega Forest is the most species-rich known forest in Kenya with a huge number of rare and endemic animals and plants (KIFCON, 1994).

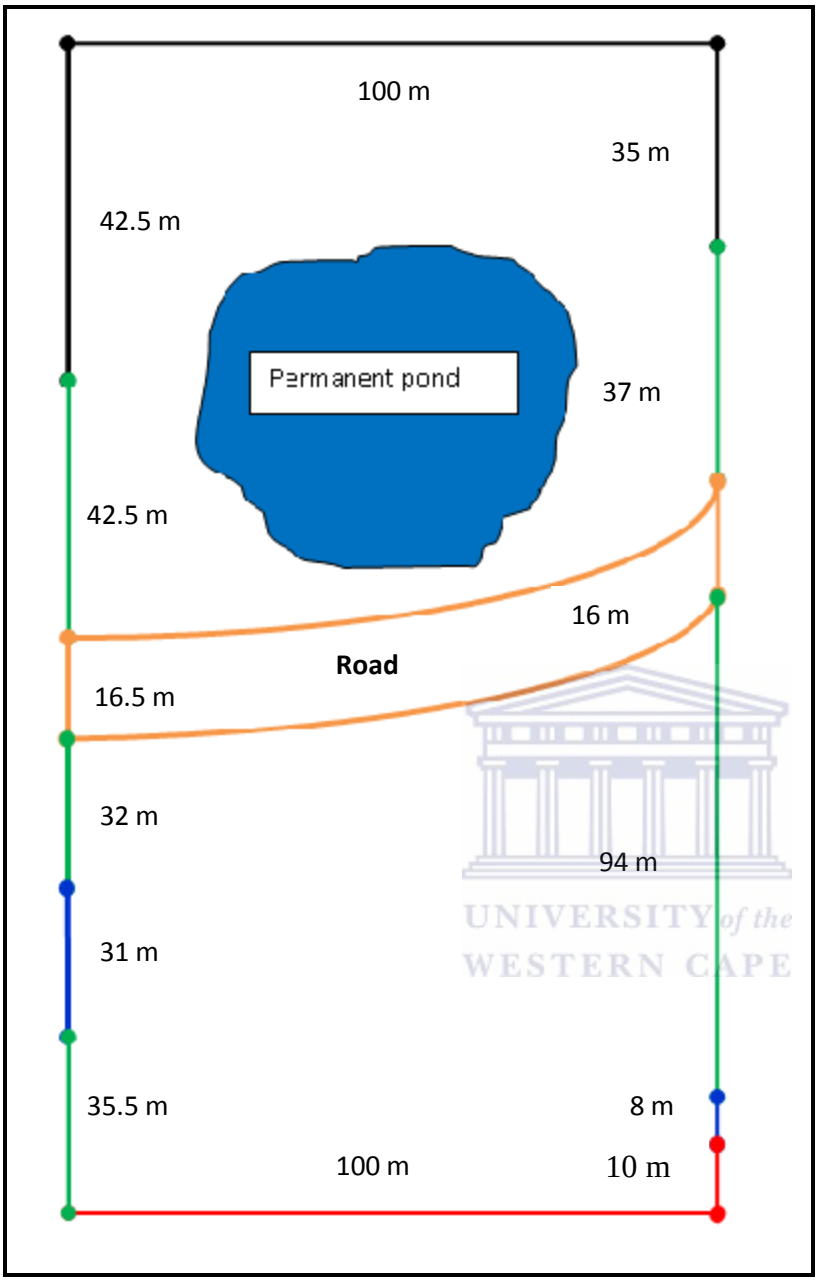
2.3.2 Transect setup

The transect runs across both primary and secondary forest. A permanent pond is enclosed in the area of the transect (figure 2.3). The transect line cuts across swampy areas with temporary streams. There are five vegetation sectors namely; primary forest cover, secondary forest made of thicket and bushes, swampy forest, swampy grassland and the road.

The primary forest segment cut across undisturbed forest part made of tall trees which formed thick canopy with little undergrowth plants and poor light penetration. In contrast the secondary forest comprised of a disturbed forest which was later reforested. This section had many undergrowth plants and shrubs with the main plant being *Dracaena fragrans*. This part of the transect was more open to light penetration as compared to the primary forest. An all-weather-murram road cut across two sides of the transect. This formed the road segment which had little vegetation (mainly grasses) as it was occasionally graded and was sandwiched between two secondary forest patches. The swampy forest had a vegetation cover similar to that of the secondary forest though having more grass. In addition there are swamps and slow flowing streams which empty into the neighbouring swampy grassland. As the name suggests the swampy grassland was made up of grasses and reeds growing on a swamp but has no tree cover (Fig 2.4).

A rectangular transect whose sides measured 600 m in total was set at Buyangu Hill. A point was randomly selected as the start (and end) of the transect. From there it ran 200 m to the north then turned 100 m to the west. From there again 200 m southward and finally 100 m east back to the starting point. The sides of the transects were demarcated by clearing vegetation along the measurements. The coordinates of the transect corners (in decimal degrees) are: 0.3528957 E,

34.8649880 S, 0.3522305 E; 34.8655512 S; 0.3515330 E, 34.8637274 S and 0.3509644 E, 34.8643764 S.



Legend

= Primary forest
= Secondary forest
= Swampy grassland
= Road
= Swampy forest

Figure 2.3. Schematic representation of the transect

Figure 2.4. Vegetation patches along the transect; left to right secondary forest, swampy grassland, swampy forest, road, primary forest canopy and the permanent pond. (Primary forest canopy photos courtesy of Henning Todt)

2.3.3 Specimen and call inventory

Using literature (Duff-Mackay, 1980; Köhler *et al.*, 2005; Lötters *et al.*, 2004; Loveridge; 1957) and museum collections in the National Museums of Kenya, Nairobi (NMK), California Academy of Sciences, San Francisco (CAS), Alexander Koenig Research Museum of Zoology, Bonn (ZFMK), a preliminary species list data for the Kakamega species was compiled (Table 2.1). This was followed by intense, purely opportunistic field surveys to complete the species list for the study area. Voucher specimens were collected and frog advertisement calls were recorded. This was undertaken by different individuals in the BIOTA E 08 resulting in a list of the frogs found in Kakamega Forest and their calls.

Voucher specimens were collected and preserved in 70% alcohol and deposited at NMK and ZFMK. Advertisement calls were recorded using a digital Sharp MD-SR 70 recorder and a Sennheiser Mc-66 Uni-directional microphone and later analyzed. The calls and specimens are described in Köhler *et al*. (2005), Lötters *et al*. (2004, 2006), and Schick *et al*. (2005). The frog calls were digitized and a copy of the compact disc with the calls was deposited in NMK and the University of Mainz (UM). The voucher specimens are as shown in Appendix 6.1.

I spent the period between mid-March and mid-April 2002 in the field learning to apply both visual and acoustic methods in the monitoring exercise. This entailed listening to previously recorded calls and later learning them in the field while seeing the actual specimens. Part of the training also involved encoding the specimen and part of the weather data into a GPS receiver and application of the program G7ToWin.

2.3.4 Transect walks

Transect walks were carried out for two consecutive days every two weeks. They were performed both during the day and at night. Day walks were carried out at midday beginning anytime between 12.00 and 13.00 hrs local time while night transects started between 20.00 and

20.30 hrs. The length of time spent was proportional to the number of frogs found. On average the walks lasted an hour during the day and two hours at night. The walks were carried out between March 2002 and December 2005.

A combination of standardized visual encounter surveys (SVTS) and standardized acoustic transect samplings (SATS) was employed to locate frogs along the transect band as described by Heyer *et al.* (1994); Rödel & Ernst (2004) and Veith *et al*. (2004). Frogs were observed within one metre on both sides of the transect (making a total of two metres). Males were also identified through their advertisement calls and those falling into the transect band up to 12.5 m on the right and left were recorded. In tropical rainforests including West Africa, this method has been successfully employed (Rödel & Ernst, 2004; Veith *et al*., 2004).

All specimen data were recorded with a GPS receiver (Garmin XL 12) as waypoints, which were encoded as illustrated in Table 2.2. For example: M01A1S would be representing a male frog, the first counted frog which was an adult. The frog was caught and was sampled when the sky was clear. All specimens were marked using a pair of scissors which was sterilized using a lighter on the second toe of the left foot. This helped to eliminate possibility of the specimens being recorded twice. Recaptured specimens were not considered in the analysis. The waypoint data included the GPS position where the specimens were found which was used to show the location along the transect line where the specimens were found.

Table 2.2. List of the GPS waypoint characters and representation of each character and the symbols used

Character	Feature denoted	Symbols applied
First	Sex	Male (M), Female (F), Unknown (U)
Second and third	Specimen/frog number	01 to 99
Fourth	Age	Adult (A), Juvenile (J)
Fifth	Sampling method	Caught (1), Observed (2), Heard (3), Recapture (4)
Sixth	Weather conditions	Clear sky (S), Rainy (R), Cloudy (C)

The data were downloaded to a computer using the program G7ToWin Version A.00.200f © C.R. Henderson, 1997-2006. The program was downloaded from http://www.gpsinformation.org/ronh/g7towin.htm. The same program was used to convert the data into Excel format for further processing.

2.3.5 Weather data

Monthly rainfall data were obtained from BIOTA subproject E03 (Regeneration of tropical upland trees - spatio-temporal dynamics of feed-back processes) from 2002 to 2005. Air temperature (taken in the road segment) was taken at the beginning and end of the walks at one metre above the ground using a Greisinger GFTH 95 thermometer to give the mean air temperature during the day (maximum) and night (minimum) walks. The mean air temperature and mean rainfall per month were compared to frog species activity during the entire research period. The word activity refers to the presence of frogs whether observed, caught or recorded when calling.

The mean monthly rainfall was divided into two categories: above 200 mm (high) and that below 200 mm (normal). The activity of frogs was described in relation to the amount of rainfall in the

counting month and the previous one. This gave rise to four categories of rainfall: high previously normal (HpN), high previously high (HpH), normal previously high (NpH) and normal previously normal (NpN). The total number of frogs counted was divided by the number of months sampled in each category. The data was tested for normality and equal variance but did not meet the assumptions. Therefore, non-parametric Kruskal-Wallis equivalent of one-way Analysis of Variance (ANOVA) on ranks was computed to test for differences in the activity between the four categories.

2.3.6 Specimen density (specimens per metre)

The distances of each vegetation segment were measured and coordinates taken at the beginning and end of each sector as shown in figure 2.4. Each specimen sampled was placed into a vegetation segment using the coordinates from the waypoints. The species density was calculated as: total number of specimens on each segment divided by the total length of each vegetation segment.

2.3.7 Community and species interactions

The specimens counted in the different vegetation segments were categorized into two groups: those counted when rainfall was above 200 mm (high) and those below 200 mm (normal) as in appendix 6.3 and 6.4. Specimen densities (specimens per metre) in the different forest fragments were computed for the high and normal rainfall categories. A multivariate analysis was performed on the data to determine the relationship between anuran communities and the species counted in the study. The density of some species was more than 10% of the total in each sample (referred to as dominant species). To eliminate this, the data was stabilised by square root transformation and a similarity matrix constructed using the Bray-Curtis index as suggested by Field *et al.* (1982). Using the group average sorting, the matrices were used to plot classification diagrams showing percentage similarity between samples. Similar clusters samples were superimposed onto a Multi-Dimensional Scaling plot. The statistical program PRIMER 5 was used for the analysis.

2.4 RESULTS

2.4.1 Species abundance over time

Out of the 24 frog species targeted for monitoring in Kakamega forest 14 were encountered during the transect walks. All the specimens sampled were treated equally with no separation as to whether sampled acoustically or by encounter. In total 256 walks were carried out yielding 565 specimens. February 2005 was the only month with no specimens counted. Sampling was carried out for a total of 37 months with no sampling in seven months (July 2002 to September 2002, March 2003 and January to April 2004).

More *Leptopelis mackayi* specimens were counted at the beginning of the study but the number declined later (figure 2.5). Two specimens were collected in the course of the study to describe the new species in May 2004.

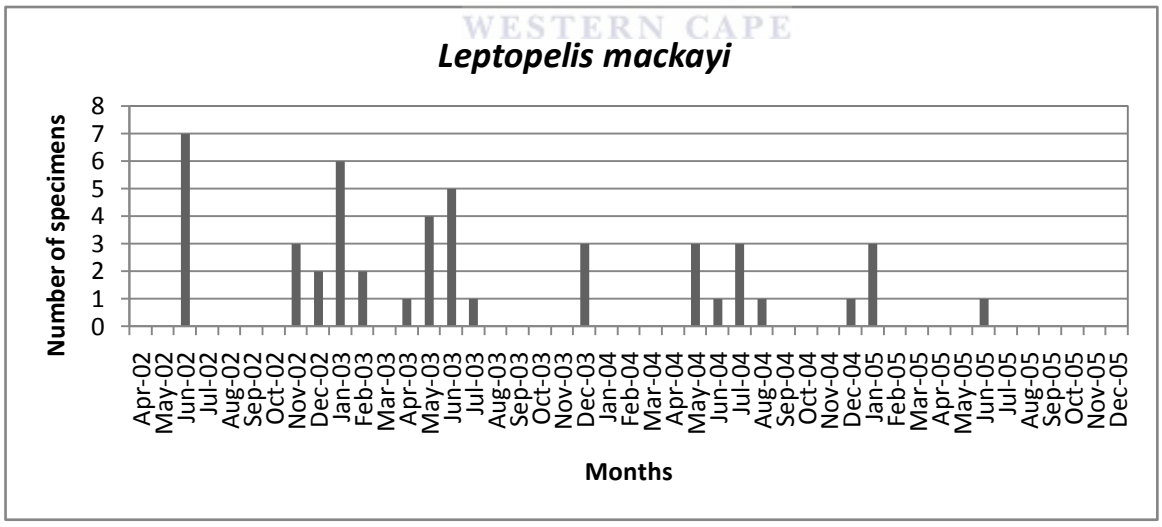


Figure 2.5. Monthly species counts of *Leptopelis mackayi*

The number of *Amietophrynus maculatus* specimens counted was double that of *A. kisoloensis* (figure 2.6).

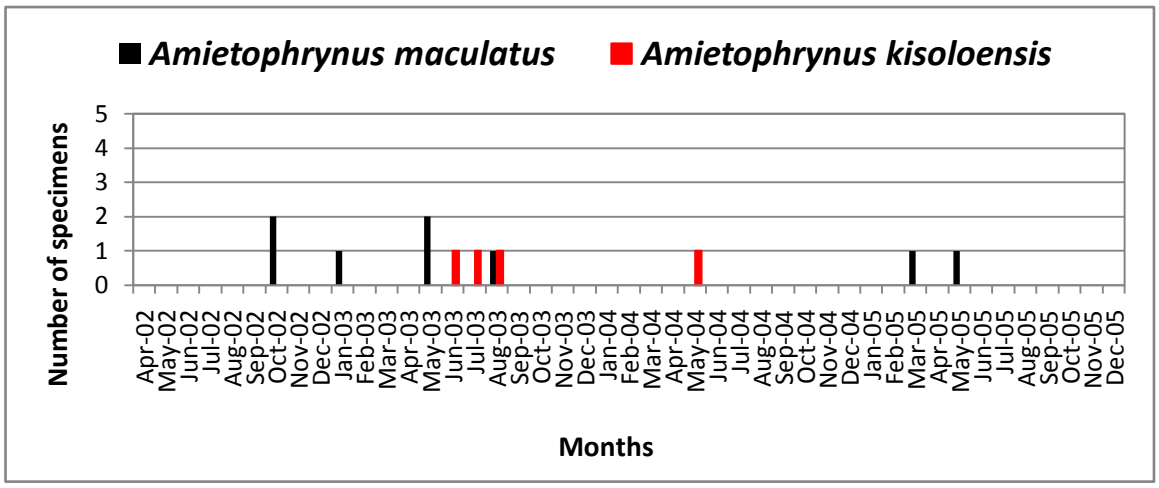


Figure 2.6. Monthly species counts of *Amietophrynus maculatus* and *A. kisoloensis*

Four specimens of *Afixalus quadrivittatus* were recorded. In May 2002 and August 2003 one specimen was sampled, while the other two specimens were counted in June 2005. One specimen of *Hypeolius acuticeps* was sampled in May 2002.

Hyperolius cinnamomeoventris was the most active species in the whole study. Its monthly specimen counts reduced as the sampling progressed (figure 2.7).

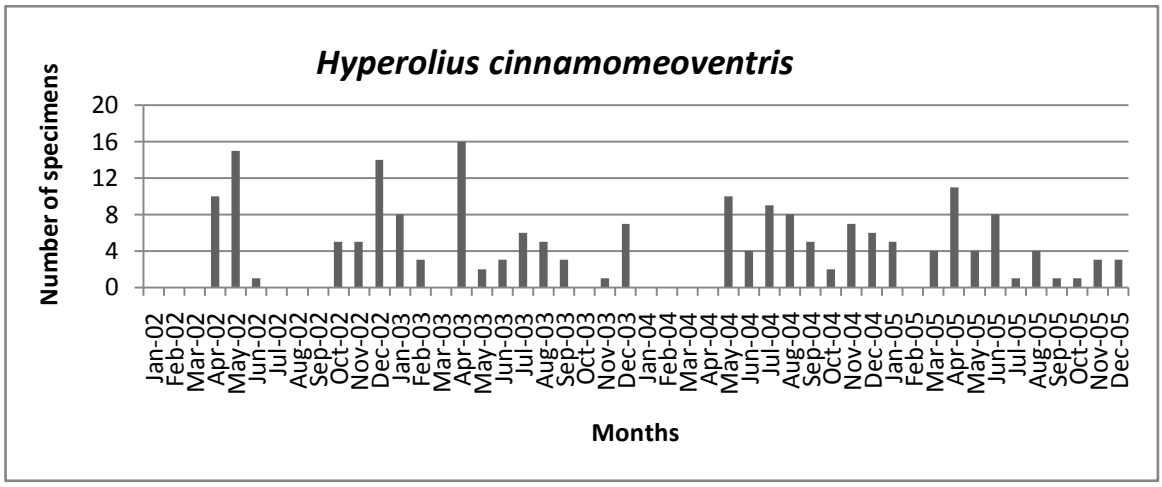


Figure 2.7. Monthly specimen counts of *Hyperolius cinnamomeoventris*

The highest number of *Hyperolius kivuensis* specimens were counted in 2002 but the species was absent in 2005 (figure 2.8).

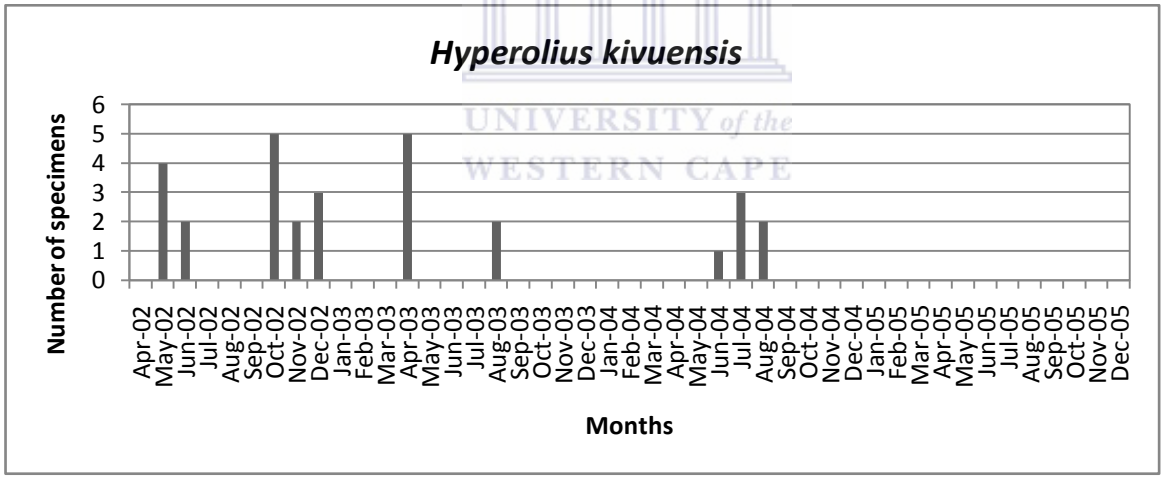


Figure 2.8. Monthly specimen counts of *Hyperolius kivuensis*

Hyperolius lateralis had the second highest number of specimens counted (figure 2.9). The activity of this specimens is high in the months of May and June though there is very little activity in 2005. On the other hand, *H. viridiflavus* was evenly distributed across the study (figure 2.10).

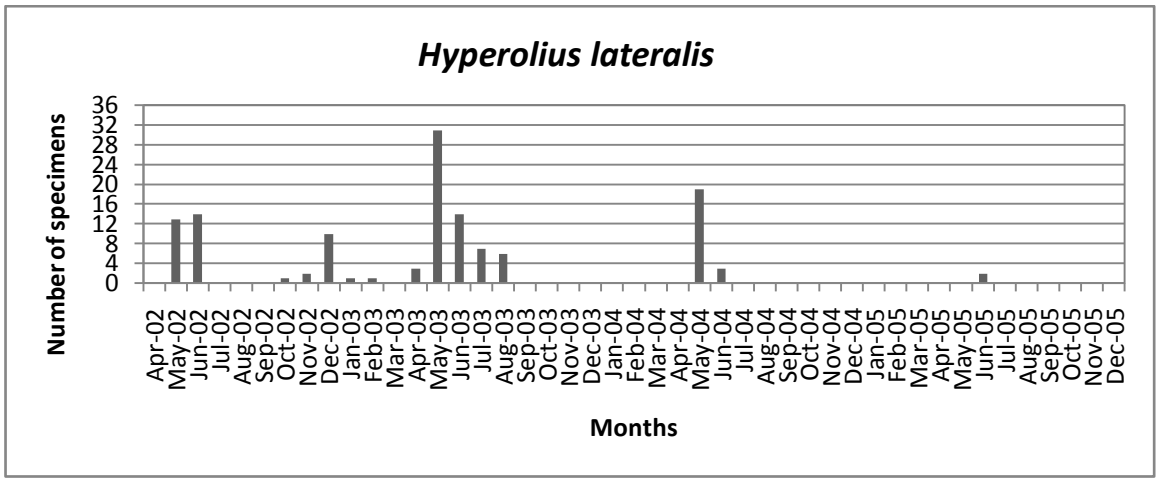


Figure 2.9. Monthly specimen counts of *Hyperolius lateralis*

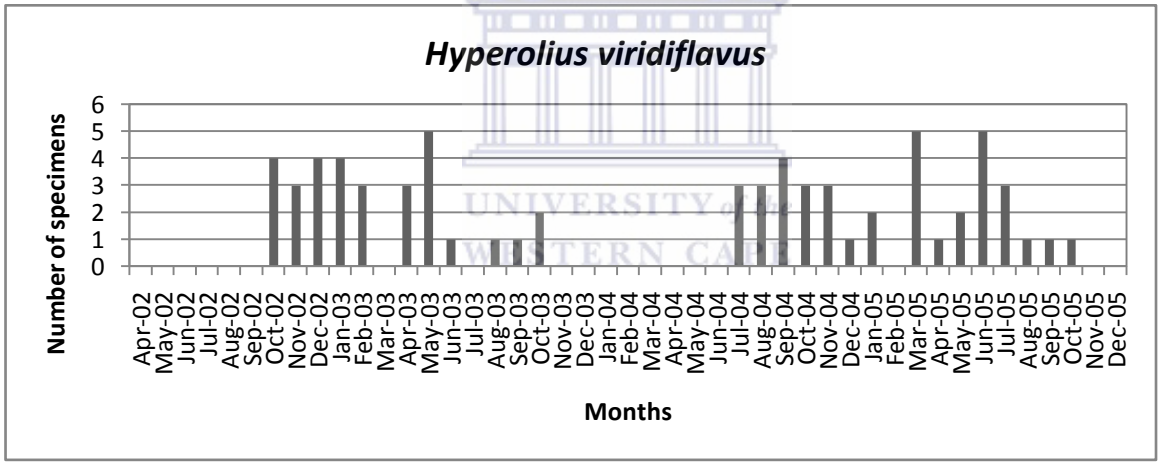


Figure 2.10. Monthly specimen counts of *Hyperolius viridiflavus*

Kassina senegalensis was sampled in 21 of the months with the highest number of species sampled in August 2004 (figure 2.11).

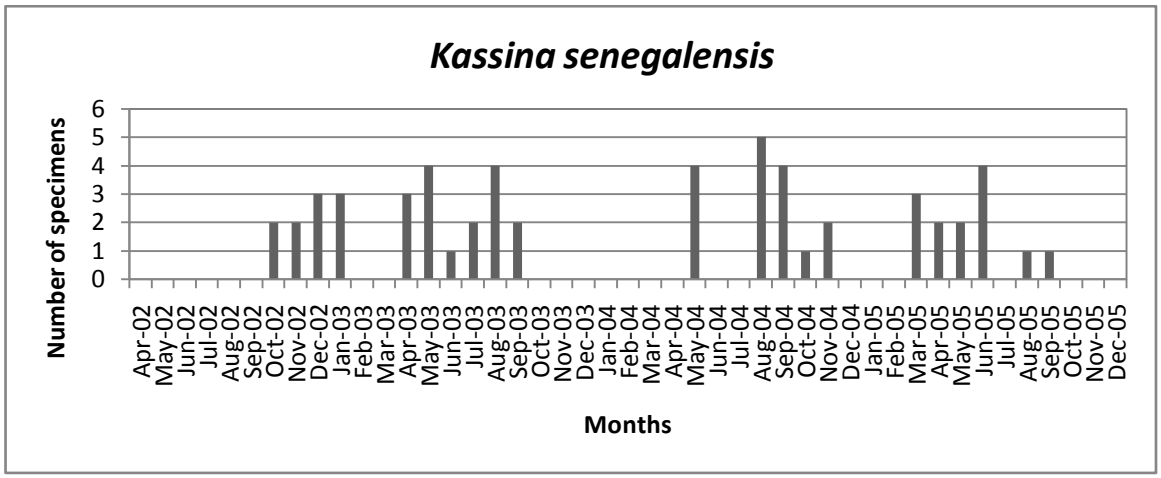


Figure 2.11. Monthly specimen counts of *Kassina senegalensis*

All the four specimens of *Phrynobatrachus natalensis* were counted in May 2002. Two specimens of *Amietia angolensis* were counted in April and May 2002. *Ptychadena mascareniensis* had all specimens counted through SVTS. In the months of October and November 2002, two specimens were counted while in February, May 2003 and July 2004 one specimen was sampled. *Xenopus victorianus* was counted four times. Three specimens were counted in August 2003, two specimens in October 2003 and June 2005 while November 2003 had only one specimen.

2.4.2 Influence of temperature and rainfall

Rainfall occurred throughout the year, even being high in months usually expected to be dry (See figure 2.12). 2003 had the highest mean rainfall, but the highest rainfall of 358.5 mm was recorded in May 2005. February 2005 had the lowest average rainfall of 27.2 mm and the highest temperature in the whole study. There was not much variation in the temperature recorded in the whole study with only five months having a high day temperature above 30^{o}C. The lowest and the highest temperatures were experienced in April 2003 and February 2005 that had temperatures of 14.8 and 39.5^{o}C respectively.

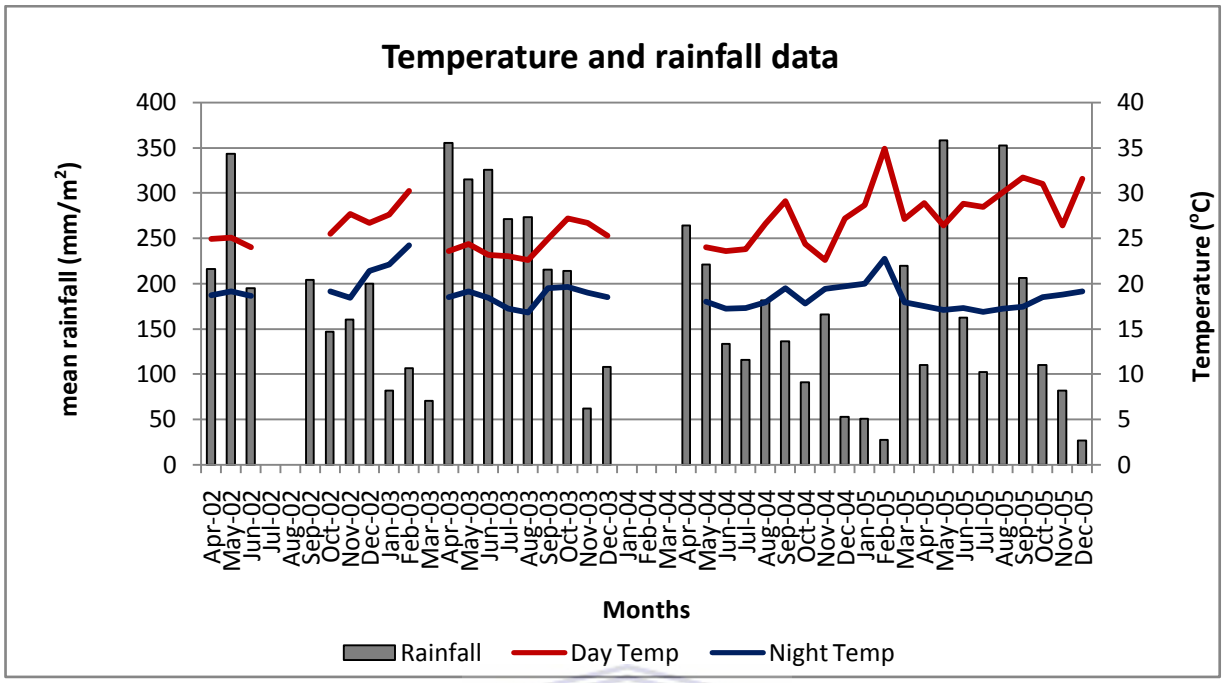


Figure 2.12. Monthly rainfall and temperature over the sampling period 2002 to 2005.

There was not much variation in the temperature recorded during the monitoring period. Also the frogs were counted in all the months except in February 2005 which had the highest recorded temperature and the lowest rainfall.

Eight species had activity in all four rainfall categories with three found in only two categories. Only three species were counted in one of each category. There were no significant differences (Kruskal Wallis ANOVA H_3=1.415, p=0.702) between the activity of frogs in the different rainfall categories..

Table 2.3. Average number of specimens counts in different rainfall categories H=High, N=Normal, p=previously)

Species name	High		Normal	
	HpN	HpH	NpH	NpN
Leptopelis mackayi	1.500	2.400	2.500	2.500
Amietophrynus kisolensis	0.250	0.800	0.200	0.500
Amietophrynus maculatus	1.000	1.500	1.000	2.000
Afrixalus quadrivittatus	0.000	0.000	2.000	0.000
Hyperolius acuticeps	0.000	1.000	0.000	0.000
Hyperolius cinnamomeoventris	8.571	5.000	4.857	4.928
Hyperolius kivuensis	4.000	3.000	1.500	3.000
Hyperolius lateralis	10.667	14.200	4.200	1.500
Hyperolius viridiflavus	3.000	2.430	2.750	2.909
Kassina senegalensis	2.667	1.000	2.500	2.867
Phrynobatrachus natalensis	0.000	4.000	0.000	0.000
Xenopus victorianus	0.000	2.500	1.500	0.000
Ptychadena mascareniensis	0.000	1.000	0.000	1.667
Amietia angolensis	1.000	1.000	0.000	0.000
Total	32.655	39.83	23.007	21.871

2.4.3 Specimen density (Specimens per metre)

Nine species were sampled in the secondary forest while eight were counted in the primary forest, swampy forest and the swampy grassland vegetation segments (Table 2.4). Only seven species were counted in the road segment. The highest number of specimens were recorded in the in the swampy grassland (see Appendix 6.2). *Hyperolius cinnamomeoventris*, *H. kivuensis* and *H. viridiflavus* were found in all the vegetation segments. Activity of *Leptopelis mackayi* was high on one side of the transect (figure 2.13 a). *Hyperolius lateralis* and *H. cinnamomeoventris* have their activity concentrated on the swampy grassland as compared to the other species.

There were no significant differences in the specimen densities of species found in the different vegetation fragments (Kruskal Wallis ANOVA H_4 =2.906, P=0.574).

Table 2.4. Specimen densities in different vegetation types

Species	Primary forest	Secondary forest	Swampy forest	Swampy grassland	Road
Leptopelis mackayi	0.000	0.017	1.012	0.018	0.000
Amietophrynus kisoloensis	0.000	0.009	0.000	0.000	0.061
Amietophrynus maculatus	0.005	0.0216	0.000	0.000	0.061
Afrixalus quadrivittatus	0.005	0.000	0.074	0.000	0.000
Hyperolius acuticeps	0.000	0.000	0.000	0.009	0.000
Hyperolius cinnamomeoventris	0.064	0.0862	0.346	1.327	0.246
Hyperolius kivuensis	0.005	0.0259	0.247	0.091	0.061
Hyperolius lateralis	0.059	0.0862	0.222	0.791	0.000
Hyperolius viridiflavus	0.037	0.129	0.196	0.182	0.123
Kassina senegalensis	0.086	0.034	0.196	0.000	0.707
Phrynobatrachus natalensis	0.000	0.000	0.000	0.000	0.123
Xenopus victorianus	0.005	0.0043	0.148	0.000	0.000
Ptychadena mascareniensis	0.000	0.000	0.000	0.064	0.000
Amietia angolensis	0.000	0.000	0.000	0.018	0.000
Total	0.266	0.4132	2.441	2.500	1.382

2.4.4 Community and species interactions

A cluster analysis at 55% Bray-Curtis similarity level (figure 2.13) reveals the anuran communities in the different vegetation segments can be classified into four groups (figure 2.14). The composition of the communities in the rainfall below and above 200 mm was similar. The secondary forest and primary forest species composition was similar in regardless of the amount of rainfall. Primary forest community is more similar to that of the secondary forest when the amount of rainfall is high.

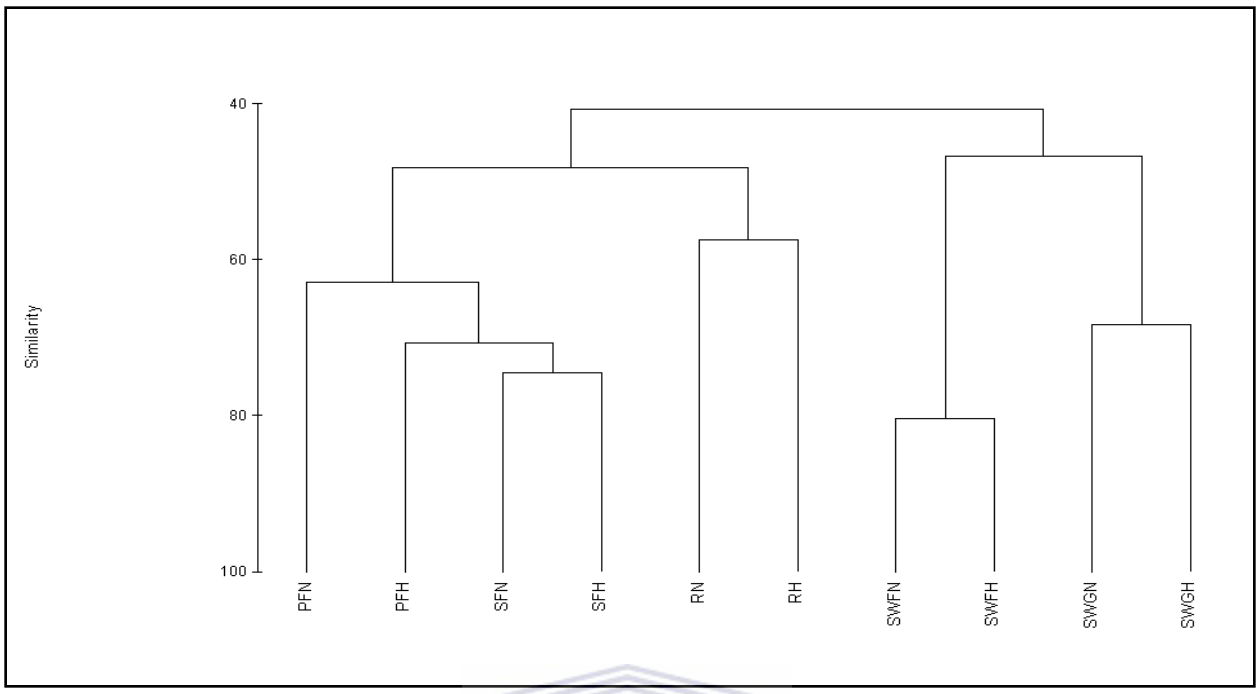


Figure 2.13. Dendogram for hierarchical clustering (using the group-average linking) for the frog communities found in the different forest fragments (PF=Primary forest, SF=Secondary forest, SWF=Swampy forest, SWG=Swampy grassland, R=Road, H=Rainfall above 200 mm and N=Rainfall below 200 mm)

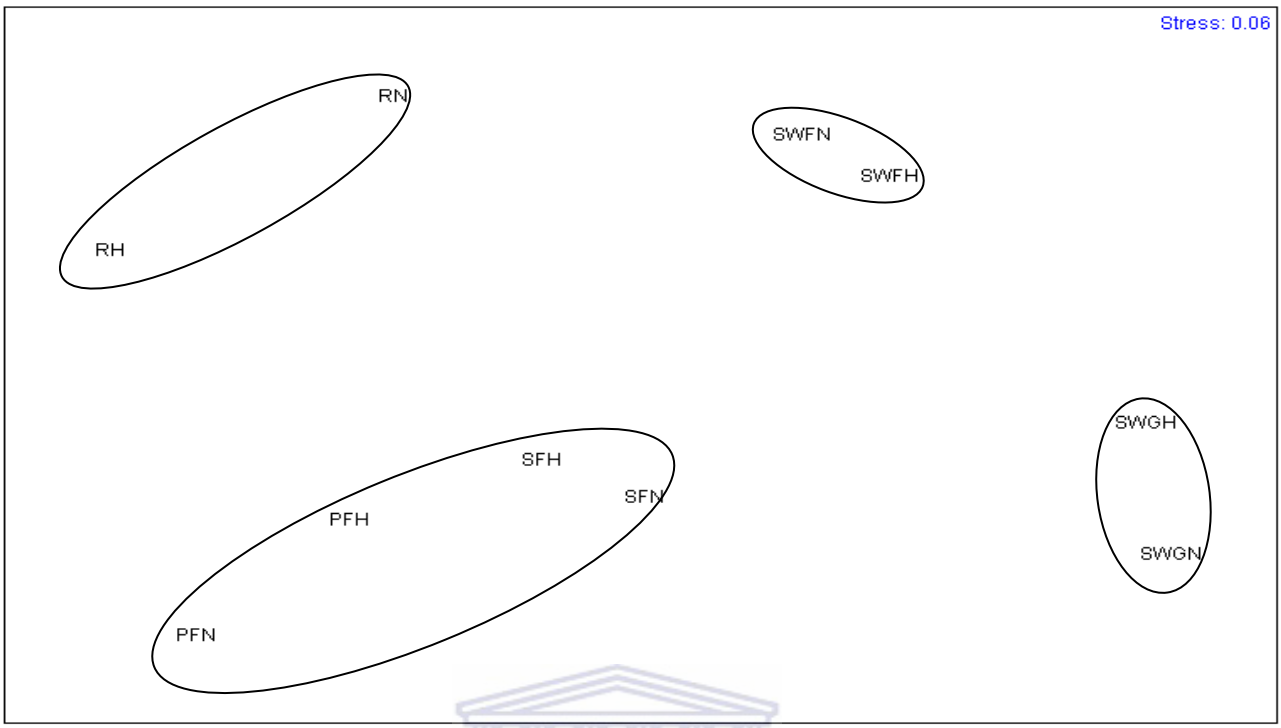


Figure 2.14. Two-dimensional Multi-Dimensional Scaling (MDS) plot (using the group-average linking) for the frog communities found in the different forest fragments

A cluster analysis of the species (figure 2.15) reveals that there are six groups with a 40% Bray-Curtis similarity. (figure 2.16). All the species were 8.6% similar with *Ptychadena mascareniensis*, *Amietia angolensis* and *Phrynobatrachus natalensis* forming a group of their own.

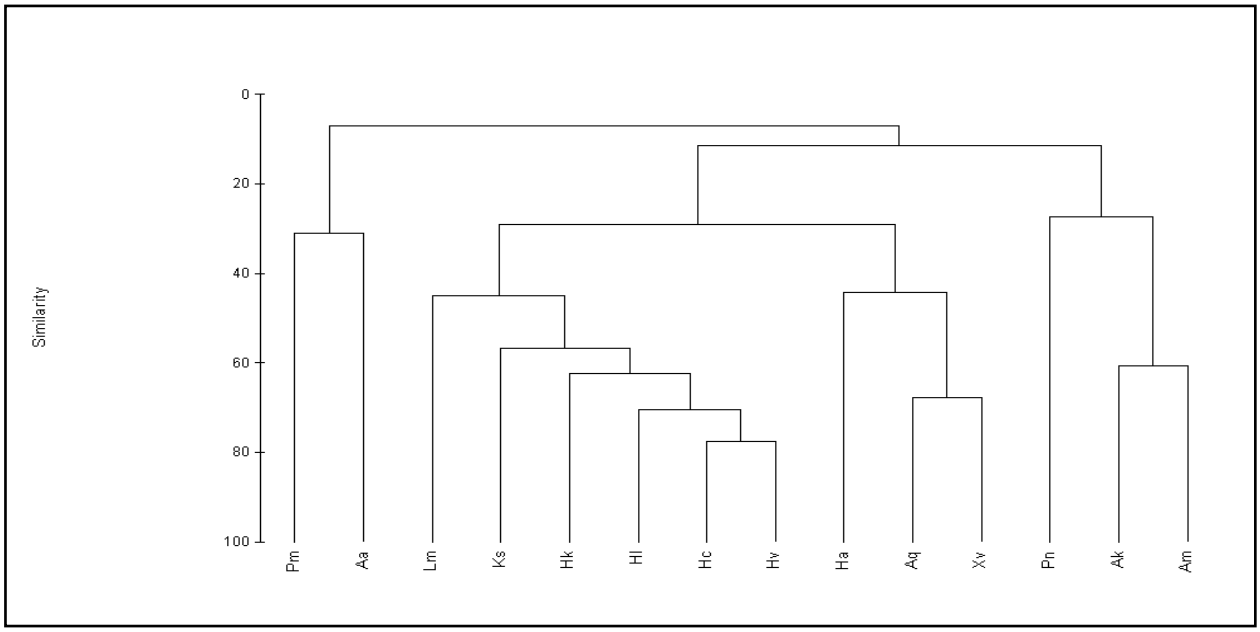


Figure 2.15. Dendogram for hierarchical clustering (using the group-average linking) for the species counted in the different forest fragments (Lm=*Leptopelis mackayi*, Ak=*Amietophrynus kisoloensis*, Am=*Amietophrynus maculatus* Aq=*Afrixalus quadrivittatus*, Ha=*Hyperolius acuticeps*, Pn=*Phrynobatrachus natalensis*, Hc=*Hyperolius cinnamomeoventris*, Hk=*H. kivuensis*, Hl=*H. lateralis*, Hv=*H. viridiflavus*, Ks=*Kassina senegalensis*, Xv=*Xenopus victorianus*, Pm=*Ptychadena mascareniensis*, Aa=*Amietia angolensis*)

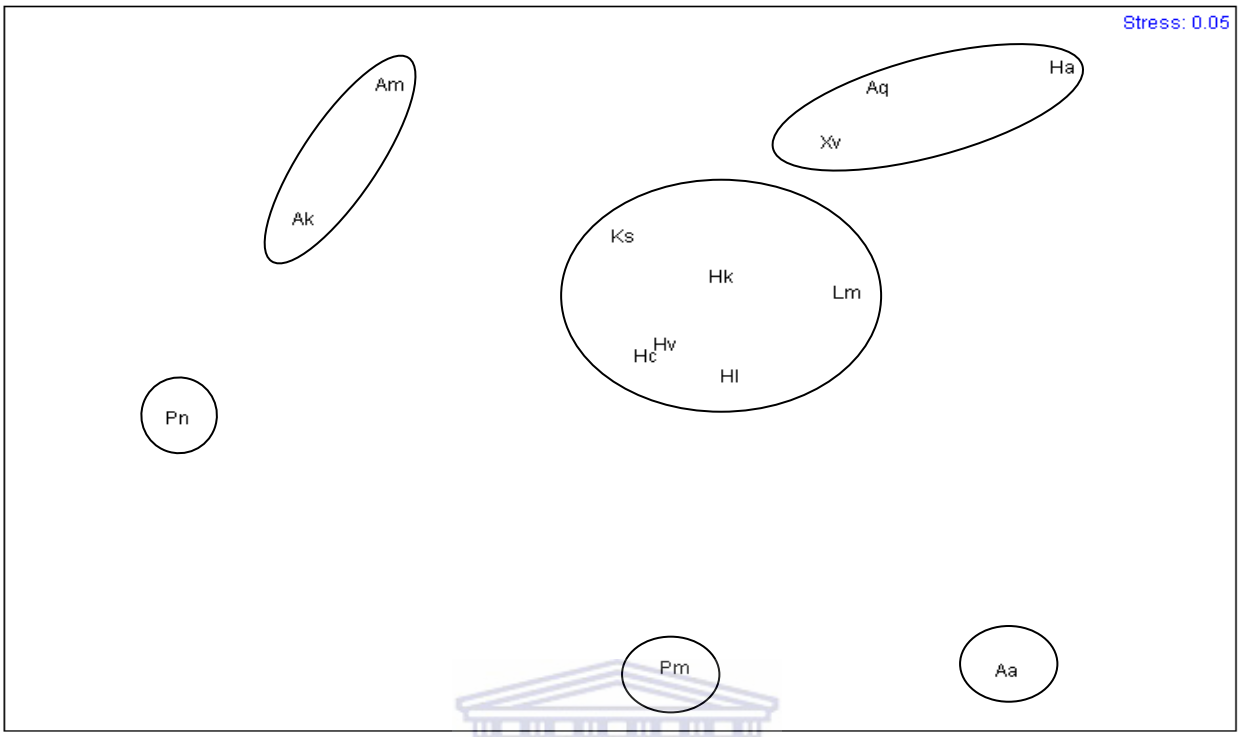


Figure 2.16. Two-dimensional Multi-Dimensional Scaling (MDS) plot (using the group-average linking) for the species counted in the different forest fragments

2.5 DISCUSSION

2.5.1 Species abundance over time

Köhler *et al.* (2006) recorded the activity of *L. mackayi* between April and September but I recorded this species throughout the year except in March and September. The reduction in the number of specimens of this species could be as a result of continued degradation of the forest section where *L. mackayi* was found. Two specimens that were used to describe the species were collected in this section (Köhler *et al*., 2006*).*

Lötters *et al.* (2004) recorded the activity of *Hyperolius cinnamomeoventris, H. kivuensis and H. viridiflavus* to be between March and October while that of *H. lateralis* to be between April and June. In this study I found *H. cinnamomeoventris* and *H. viridiflavus* to be active throughout the year. I also found that the activity of *H. kivuensis* extending to December while *H. lateralis* was present throughout the year except in March and September.

The extension of the activity of these species might have been occasioned by differences in the sampling areas and time spent. While I concentrated on the transect, Lötters *et al.* (2004) used data from the transect, the permanent pond within the transect (where most of the study was done) and other ponds. Lötters *et al.* (2004) collected data over a one year span while my data was recorded over a period of 37 months.

Razzetti & Msuya (2002) recorded that *Kassina senegalensis* breeds between May and June in Arusha National park, which has a higher altitude than Kakamega. From my findings the activity of calling the species extended beyond was in all the months except February as in figure 2.11.

P. mascareniensis tendency to escape when approached may have led to fewer specimens being counted (Veith *et al.* 2004). Few specimens of *Hyperolius acuticeps*, *Phrynobatrachus natalensis* and *Amietia angolensis* were recorded therefore not enough to make any discussion on it.

2.5.2 Influence of temperature and rainfall

As temperatures rise there is increased evaporation thus moisture loss which may lead to dehydration. On the other hand, temperature is known to affect calling of male frogs. Blair (1961) observed that increase in temperature has a positive effect on anurans calling. However, at a certain critical temperature, activity of amphibians ceases (Blair, 1960, 1961; Byrne 2002; Stewart, 1995).

The activity of amphibians in Kakamega Forest is influenced to a larger extent by rainfall than temperature. There was not much variation in temperatures recorded in the study. Notably, February 2005 that had the highest temperature and the lowest amount of rainfall, but no specimens were counted.

Rainfall is known to influence the activity of amphibians (Bertoluci & Roudrigues, 2002; Blair, 1960, 1961; Bowker & Bowker, 1979; Saenz *et al.*, 2006; Stewart, 1985, 1995). In Java (Indonesia), Church (1961) found that though rainfall initially influences the breeding of *Duttaphrynus melanostictus*, it was no longer a determining factor after the process had begun. Kam *et al*. (1998) found the activity of *Huia swinhoana* in Taiwan to be higher in drier months than in wet months.

In South Africa the activity of the giant bullfrog (*Pyxicephalus adspersus*) is determined by the amount of rainfall in the preceding days. The activity of this species is prompted by rainfall

above 30 mm but not persistent light rainfall (Du Preez & Cook, 2004). The movement of European grass frogs to breeding sites is triggered by rainfall (Obert, 1976) but has no bearing on the activity thereafter.

Whitmore (1990) describes a tropical rainforest as not having much variation in temperature and rainfall being spread throughout the year. Kakamega Forest being a tropical rainforest does not have much variation in temperature, except some extreme temperature. It was only in the month with extreme temperature (February, 2005) was there no counts of frogs. On the other hand rainfall was spread through the year with only two months with rainfall below 50 mm. Therefore the activity of frogs is not dependent on the amount of rainfall but on others factors. There were frog counts in all the sampling months except in the month with the lowest rainfall.

2.5.3 Specimen location and specimen density

The swampy forest accounted for 84.8% of the total specimens of *Leptopelis mackayi* sampled. The locality is an ideal breeding site for *L. mackayi*, with trees, which the species uses for calling and available shallow water for tadpole development (Köhler *et al.,* 2006). Location of the specimens in the swampy grassland and secondary forest is due to their proximity to the swampy forest.

The two bufonids, *Amietophrynus kisoloensis* and *A. maculatus* had more specimens sampled in the secondary forest than other segments. This segment is near the pond and the road. On the road leading to the transect there were puddles full of bufonid eggs. This is an indication that the frogs found on the road may be moving towards their breeding sites. In the primary forest, the frogs would be migrating toward the ponds where breeding and oviposition would take place.

The location of *Afrixalus quadrivittatus* is dependent on the prescence water in which is near the primary forest with a pond and in the swampy grassland.. *Hyperolius kivuensis, H. lateralis, H.*

cinnamomeoventris and *H. viridflavus* call on vegetation and lay eggs attached to vegetation in water (Lötters *et al.*, 2004; Schiøtz, 1999) which is provided by streams flowing into the grassland. This explains their abundance in the swampy grassland. All the four species and *H. acuticeps* were found in the expected forest fragments as defined in Lötters *et al*. (2004) with most of them being found in the swampy grassland.

Amietia angolensis was found in the typical habitat described in Channing & Howell (2006). All *Ptychadena mascareniensis* specimens were found in the swampy grassland. This is a good site for this species to camouflage as using its colour which blends well with the vegetation.

Xenopus victorianus is a fully aquatic species which explains the location in the swampy forest. This section has streams which provide a good habitat for the species. The two specimens found in the primary and secondary forests were migrating perhaps towards the permanent pond or the swampy grassland. Migration in search of water bodies is one of the adaptations of this species (Tinsley & McCoid, 1996).

The swampy grassland is the only open section of the transect. The segment is in direct contact with abiotic factors such as rainfall and sunlight as it has no tree cover. Insects perch and feed on the grass, while some are attracted by the dead vegetation. Streams empty into this section providing moisture. Most specimens were counted in this habitat. It provides a good breeding ground for frogs with the grass which some species perch on when calling. Use of cryptic colouration is one of the attributes of amphibians (Norris & Lowe, 1964) with shades of colour provided by live and dead plant material. The section has more light reaching the specimens as illumination improves the ability of amphibians to detect and avoid predators (Tuttle & Ryan, 1982, Tuttle *et al.*, 1982).

The swampy forest segment despite covering a short length of the transect had a high specimen density with a high number of specimens. Eight species, all of which are tree frogs, were counted in this segment, which is lowest of all the segments. This is an ideal breeding habitat for the species (Köhler *et al*., 2006; Lötters *et al*., 2004 Schiøtz, 1999). The segment is more open than the primary forest in terms of light and heat penetration. There were streams flowing that provide good breeding sites.

Primary forest had the lowest species density as compared to all other segments despite being the second largest segment and a high number of species. Thick canopy cover inhibits the growth of smaller plants which are essential for most anurans and therefore not a good habitat for most of the anurans. Frogs are exothermal animals and their activity is affected by external temperature (Duellman & Trueb, 1986). Heat from the sun does not reach the ground due to the thick tree canopy. Frogs found in this segment are those migrating, on the few low growing plants or due to the segment proximity to the pond. Terrestrial and arboreal species were found in this segment with one aquatic species counted migrating to the pond in the proximity.

Secondary forest was the largest segment of the transect with the highest number of species and the second largest number of specimens. It forms part of disturbed and reforested vegetation with shrubs and undergrowth plants. *Dracaena fragrans* is the main undergrowth plant, which is a good hiding place for tree frogs and is able to trap moisture. A mixture of arboreal, ground dwelling frogs and one aquatic species were found here. The arboreal and the terrestrial species presence is a result of habitat preference but the aquatic *Xenopus victorianus* was migrating towards water bodies near this segment. Light and heat penetration is adequate and provides for shade to prevent water loss for the frogs. The different patches with this type of vegetation type are near swampy grassland, swampy forest or the permanent pond.

The road segment has little vegetation, which is occasionally removed when the road is graded. This low growing vegetation is good for ground dwelling individuals to hide. The segments are

located between primary forest patches and but do not have much vegetation cover. This translates into improved light and heat penetration but also has high evaporation. Proximity to the pond and species crossing over between forest fragments influences the location of specimens. Both arboreal and terrestrial frog species were found in this segment.

2.5.4 Community and species interactions

Primary forest community composition is more similar to that of the secondary forest when there is increased amount of rainfall. Increase in the amount of rainfall translates to increased moisture, which leads to more activity from the neighbouring secondary forest segments and the permanent pond, which is near this segment. All the other segments are similar in their composition or change slightly when species are counted when there is normal and high rainfall. However, the number of species counted in the road segment changes considerable between the two rain categories (Table 2.15). When there is increased amount of rainfall, pools form on the road, which are good breeding sites for some species.

The species counted in this study can be classified into five habitats according to their similarities in habitat they were sampled. *Ptychadena mascareniensis* and *Amietia angolensis* were sampled only in the swampy grassland, which is located at the edge of the forest, the ideal habitat as described by Channing & Howell (2006). Their placement in the different groups may be as a result of fewer specimens counted rather than differences in habitats they occupy.

Leptopelis mackayi, *Kassina senegalensis Hyperolius cinnamomeoventris*, *H. lateralis* and *H. viridiflavus* have more similar habitat characteristic types. All except *Kassina senegalensis* (semi-terrestrial) are arboreal species that are found in the forest (Channing & Howell, 2006; Köhler *et al*., 2006; Lötters *et al*., 2004). *Kassina senegalensis* is mostly found near water sources (Lötters *et al*., 2004) which is provided by the permanent pond which is near the transect

segments where the species was recorded. *Hyperolius cinnamomeoventris* and *Hyperolius viridiflavus* are more similar than all the other species in their habitat preferences.

The two members of the family bufonidae *Amietophrynus kisoloensis* and *A. maculatus* have similar characteristics of the habitats they occupy. Both are terrestrial species found in almost similar habitats (Channing & Howell, 2006). *Afrixalus quadrivittatus, Xenopus victorianus* and *Hyperolius acuticeps* share similar characteristics. Only a single count of *H. acuticeps* was made in the study. *Afrixalus quadrivittatus* and *Xenopus victorianus* were counted in similar habitats.

2.5.4 Species absent

Ten species were not recorded through transect walks even though they are expected in the Kakamega Forest. *Leptopelis bocagii, Phrynobatrachus graueri* and *Hydrophylax albolabris* had been recorded in the forest in previous years (Lötters *et al.,* 2007, Veith *et al*., 2004) but were notably absent in the transect and in the surrounding forest patches .

Four members of the family Ptychadenidae: *Ptychadena anchietae, P. oxyrhynchus, P. porosissima* and *P. taenioscelis* were also not recorded in the study. Their ability to camouflage and escape when approached is another feature that may have led to them not being sampled.

Afrixalus osorioi, Phrynobatrachus minutus and *Hoplobatrachus occipitalis* were also not recorded. This may be as a result of the transect not cutting through the habitats where they are found. *Hoplobatrachus occipitalis* is found in aquatic habitats (Channing & Howell, 2006; Lötters *et al*., 2007) which was not found along the transect path. *Afrixalus osorioi* was recorded in the permanent pond which is in the vicinity of the transect during the study. The species is found in the forest and forest edges (Lötters *et al*., 2006; 2007). Since it was not found in the transect I suggest that this species' habitat is restricted to near water bodies.

2.6 Summary

- Out of the 24 species targeted for monitoring only 14 were encountered in the study most of which were member of the family Hyperoliidae. *Leptopelis mackayi, Hyperolius lateralis, H. kivuensis, H. viridflavus, H. cinnamomeoventris* and *Kassina senegalensis* had their period of activity recorded previously in other studies extended.

- .Activity of frogs was not affected by temperature and rainfall as the there were not big variations in temperature and on the other hand rainfall in Kakamega Forest was spread throughout the year.

- Most specimens were counted in the swampy grassland, which also had the highest species density. The primary forest had the lowest number of specimens and specimen density as compared to the other segments.

- The amphibian community in Kakamega Forest can be divided into six groups of species that have similar habitat characteristics.

Chapter 3: Influence of abiotic factors on when and how long to conduct anuran research

3.1 Introduction

Monitoring is an expensive venture in terms of money and time and therefore it requires adequate planning (Doan, 2003; Greenwood, 1996; Heyer *et al.*, 1994). Doan (2003) notes the need to come up with methods that achieve research objectives efficiently in tropical rainforests. One of the fundamental question that a researcher asks is how long they would need to sample in order to get quality data. Greenwood (1996) notes the underlying importance of placing quality of work ahead of costs. Nevertheless, this is a challenge to many scientists as in most cases they are not the financiers of the projects. They find themselves with the problem of terminating their research before it can be conclusive or finishing in the minimum time possible. To avoid this, researchers need to come up with findings on how long different surveys can be can be undertaken without comprising their quality.

3.1.1 Methods for amphibian monitoring

One of the Amphibian Conservation Action Plan (ACAP) strategies is to have more research addressing the problem of amphibian decline and extinctions. This involves coming up with methods that are able to predict the trends of amphibian population status (GAA website, 2007). This calls for development of methodologies that are efficient in order to have a timely response in addressing the problem (Rödel & Ernst, 2004). This has to be done without compromising results.

The method to apply in monitoring of amphibians is dependent on the goal(s) of the study, duration of the study, personnel available and the cost to conduct the survey (Heyer *et al.*, 1994). Transect sampling is an ideal method for long term amphibian monitoring as it is able

to combine different sampling methods and provide for standardisation of methods (Jaeger, 1994; Rödel & Ernst, 2004; Veith *et al*., 2004). Chapter 2 describes the method in detail.

3.1.2 Species richness estimation

Species do not live in an ecosystem alone but in a community, therefore it is important to measure how species interact with each other. One of the measures is species richness, which is a measure of how many species are present in a community (Colwell, 2006; Krebs, 1998). For large communities it is not possible to count each individual specimen due to the complexity of species communities (Chao, 2005; Krebs, 1998; Smith & Van Belle, 1984). Therefore, there is a need to use non-parametric methods to handle representative data to obtain species richness (Chao, 2005; Smith & Van Belle, 1984). Species richness estimation in a community is used to extrapolate the number of species that can be found if sampling were continued to have an infinite number of walks. This can be achieved by the use of species richness estimators (Veith *et al.,* 2004).

Several estimators have been suggested in estimating species richness and abundance (Colwell 2006). Nevertheless, there is no agreement as to which estimator is best to use (Carpentier *et al*., 1998; Chazdon, *et al.,* 1998; Smith & van Belle, 1984).

Colwell & Coddington (1994) tested the use of the Jackknife 1 & 2, Chao 1 & 2, Bootstrap, and Michaelis-Menten estimators and found that Chao 2 and Jackknife 2 gave accurate estimates for small samples. Helste & Forrester (1983) found that Jackknife has more estimator bias when applied on many samples. Chazdon *et al*. (1998) found Incidence-based Coverage Estimator (ICE) the ideal estimator and Chao 2 performing well with small samples. Jackknife 2 also performed well as an estimator. In this study, I tested Incidence-based coverage estimator (ICE), Abundance-based coverage estimator (ACE), first order Jackknife,

Chao 1, Chao 2 and Coleman rarefaction in their performance in estimating species in Kakamega forest. The formulae for all the estimators are in Appendix 6. 5.

Species observed (Sobs) is a species estimator that gives the total number of species in the total number of samples. It is used in plotting the species accumulation curves for the number of species recorded (Colwell, 2006; Magurran, 2004). Abundance-based coverage estimator (ACE) is a richness estimator that uses abundance data of species with less than ten individuals (Magurran, 2004). It estimates the overall species richness by incorporating rare species data (Chao *et al.*, 2000; Colwell & Coddington, 1994).

Incidence-based coverage estimator (ICE) is a coverage estimator utilising incidence data of species with ten or more individuals. It includes species not present in the samples observed thereby enabling the extrapolation of the total species expected in a study (Chazdon *et al*. 1998; Colwell, 2006). This is important since in most instances as it is not possible to count all the species in an area and species accumulation curves do not directly show the number of species present (Magurran, 2004). The first order Jackknife (Jack 1) is a species richness estimator, estimating the overall species richness with even species absent in samples (Burnham & Overton, 1979; Colwell, 2006; Helste & Forrester, 1983; Smith & Van Belle, 1984). The method has no assumptions when sampling in one area but in more than one area the assumption is that the samples are independent (Smith & Van Belle, 1984). It was developed to reduce bias in estimators (Chao, 2005).

Chao 1 is a richness estimator that uses abundance data. It estimates the absolute number of species in a community. The Chao 1 estimator focuses on the number of the rare species in a sample in that they carry more information about the missing ones (Chao, 2005; Colwell, 2006; Colwell & Coddington, 1994). Chao 2 is a modification of Chao 1 that uses incidence data. It estimates the total number of species including species not present in the samples (Chao *et al*. 2000; Chazdon *et al*., 1998; Colwell, 2006).

Coleman rarefaction gives the estimate of the total number of expected species and has the assumption that the individual specimens are randomly distributed (Colwell, 2006). It is useful in community sampling methods but has the disadvantage of having a high bias with a small sample (McCabe & Cyr, 2006).

Doan (2003) recommends testing of other factors influencing sampling other than VES and quadrats to come up with the most efficient technique under in different conditions. Estimator performance is affected by various parameters of VES and AES, such as the number of species counted, number of specimens and number of transect walks (Veith *et al.,* 2004). In the case of anurans, estimators' performance can be compared to other parameters like rainfall, moon phase, and time of the day, which are known to affect amphibian activity. The response of different anurans to these factors varies also amongst species even within the same community (Jaeger, 1994).

3.1.3 Influence of abiotic factors

In this study, I compared how time of the day, rainfall and cloud cover affects the sampling of frogs. In addition, I examined how the new and full moon phases affect frog activity. Rainfall is an influential factor in the biology of amphibians (Bertoluci & Rodrigues, 2002; Blair, 1960, 1961; Bowker & Bowker, 1979; Oseen & Wassersug, 2002). This is because amphibians depend on moisture for reproduction (Duellman & Trueb, 1986). Some frog species (e.g. *Anaxyrus microscaphus*) have their acoustic transmission affected by rainfall and therefore avoid it (Dorcas & Foltz, 1991). The influence of rainfall and its direct or indirect effect on the activity of anurans is an essential parameter to study.

Amphibians have permeable skins and therefore lose water through evaporation (Duellman & Trueb, 1986). Cloud cover helps reduce the effect of radiation by reduced temperatures and

reduced evaporation. This translates into water bodies not drying fast and therefore more time for breeding and development of the tadpoles (Dobkins & Gettiger, 1985; Naniwadekar & Vasudevan, 2007). In this study, I compared the activity of frogs when there is cloud cover and when the sky is clear.

The phases of the moon are known to affect the activity of amphibians. Byrne (2002) found that the activity of *Crinia georgiana was* higher towards the full moon as compared to the new moon. This is different from previous findings by FitzGerald & Bider (1974) who found that *Anaxyrus americanus* had more activity during the new moon phase as compared to the full moon phase. However, they noted that this activity could be masked by the other environmental factors such as rainfall and temperature. Phase of the moon is a good factor to consider as there is limited knowledge on this factor's influence on anurans. In this study, I tested the differences in activity between new moon and full moon phases.

Frogs can be either nocturnal or diurnal with some being active both during the day and at night (Bowker & Bowker, 1979; Duellman & Trueb, 1986; Passmore & Carruthers, 1995; Schiøtz, 1999). Visibility of specimens varies during the day and night. During the day, animals are able to detect the observer and escape, or rely on camouflage. Sampling at night involves use of a torch, which allows one to notice differences in colour patterns of frogs and vegetation when frogs attempt camouflage (Veith *et al*., 2004). At night, the weather is favourable as the temperatures are low and therefore there is reduced moisture loss, thus increased activity of anurans (Duellman & Trueb, 1986). However, most activity of anurans is influenced by rainfall and therefore most of them are active in the day during or after rains.

3.2 Aims

In this chapter I seek to determine how many transect walks are needed in order to achieve representative results under different environmental conditions. I also want to compare how the activity of amphibians is affected by moon phases and how cloud cover and rainfall affected sampling of frogs.

3.3 Methods

The study was undertaken in Kakamega forest, which is Kenya's only remnant of the once vast Guineo-Congolian forest (Fashing & Gathua, 2004; KIFCON, 1994; Köhler *et al*., 2003). It is Kenya's most rich forest in terms of biodiversity with many rare and endemic species found there KIFCON, 1994). Sampling was done using transect walks, which were repeated every two weeks. The forest and the method are described in detail in Chapter Two.

The data was filtered using Microsoft Excel for the various weather conditions and full moon days. New moon and full moon dates were determined for the days that the transect walks were carried out by considering five days to and from the day with either the new or full moon.

The results were analysed using EstimateS version 8.0.0 downloaded from http://viceroy.eeb.uconn.edu/EstimateS. Diversity settings were set to compute Chao 1 and Chao 2 using the formula as described in Chao (1987). The data were randomised a hundred times without replacement. Graphs were drawn in Excel for each estimator. To determine when sampling was most effective, I compared the number of walks needed to count 90% of the total number of species sampled under different weather conditions.

To eliminate the bias arising from the differences in number of walks the sampling index was expressed as the number of specimens sampled divided by the number of walks as in Table 3.1. The data of the different weather conditions were analysed to determine whether there were differences. This was done using a non-parametric equivalent of one-tailed ANOVA (Kruskal-Wallis ANOVA) since the data was not normally distributed for all the data. This was followed by multiple comparison tests using Tukey test between the groups. Paired comparison tests were then computed using a two-tailed Mann–Whitney U test to determine the differences between the new and full moon phase data. These tests were computed using the statistical programme SPSS version 14.

3.4 Results

In total, 256 walks were carried out accounting for 126 day and 130 night walks. All the walks yielded a total of 565 specimens; 533 during night sampling and 32 from the day counts (Appendix 6.6). Most of the walks were carried out under cloudy weather conditions while those under rainy conditions were the fewest (Table 3.1). Arboreal species accounted for 94.3% of the total specimens with the rest being terrestrial and aquatic species.

Out of the 24 species targeted for monitoring of the Kakamega frog community 14 were confirmed through transect walks. Seven known arboreal hyperoliid species were counted during the walks. Also counted were five terrestrial species, one aquatic species and one semi-terrestrial species (*Kassina senegalensis*).

Hyperolius acuticeps and *Amietia angolensis* had the lowest number of specimens (one and two respectively), while 200 specimens of *Hyperolius cinnamomeoventris* and 127 of *Hyperolius lateralis* were counted. *H.viridiflavus* was the species that was active in most of the weather conditions except when it was raining during the day.(Appendix 6.6).

All the 14 species encountered in the study were recorded during night sampling as compared to ten in the day. All the species overlapped except for *Afrixalus quadrivittatus, Hyperolius acuticeps, Phrynobatrachus natalensis* and *Amietia angolensis*, which were only counted at night. Under clear sky five specimens were sampled during the day and 69 at night. On the other hand, under cloudy conditions there were nine species during the day and 14 at night. Only two species were counted during the day when it was raining while nine were sampled at night.

There was significant difference (Kruskal Wallis ANOVA H_5=25.8, P=0.0001) in sampling in the different weather conditions under which the study was carried. More amphibians were

caught under cloudy, rainy and clear conditions at night than under any weather condition during the day, and that during the day, more amphibians were caught during cloudy conditions than when it rained or skies were clear. There was no difference in catch among night conditions and there was no difference between clear and rainy days. Only *Xenopus victorianus* had a higher sampling effort ratio during the day as compared to night sampling.

There were more specimens counted during the day when it was cloudy ($p=0.026$) than when it was raining or sunny ($p=0.1$). There were no significant differences in the number of specimens sampled when it was sunny and when it was raining ($p=0.1$). There was no significant difference in the number of specimens counted under different conditions at night ($p=0.766$).

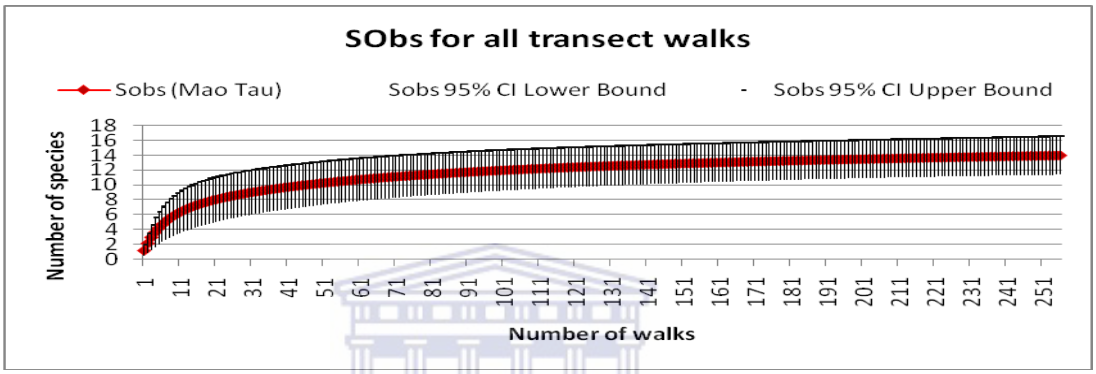
Both the new and full moon phases had equal numbers of species recorded. Both had overlapping species except *Amietophrynus maculatus, Afrixalus quadrivittatus* and *Amietia angolensis.* There was no significant difference ($p=0.963$) when sampling in the two moon phases.

Table 3.1. Summary of the sampling index (species/day) for the different species sampled under different weather conditions (D=day, N= Night)

Species name	**Clear sky (D)**	**Cloudy (D)**	**Rainy (D)**	**Total**	**Clear sky (N)**	**Cloudy (N)**	**Rainy (N)**	**Total**	**New Moon (N)**	**Full moon (N)**
Number of walks	**45**	**78**	**4**	**126**	**26**	**88**	**16**	**130**	**33**	**43**
Leptopelis mackayi	0.0000	0.0256	0.0000	0.0256	0.1154	0.4204	0.3125	0.8483	0.0909	0.4419
Amietophrynus maculatus	0.0222	0.0000	0.0000	0.0222	0.0769	0.0455	0.0625	0.1849	0.0000	0.0930
Amietophrynus kisoloensis	0.0222	0.0128	0.0000	0.0350	0.0000	0.0227	0.0000	0.0227	0.0303	0.0000
Afrixalus quadrivittatus	0.0000	0.0000	0.0000	0.0000	0.0769	0.0227	0.0000	0.0996	0.0303	0.0000
Hyperolius acuticeps	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0000	0.0114	0.0000	0.0000
Hyperolius cinnamomeoventris	0.0000	0.0512	0.2500	0.3012	1.000	1.5682	1.9375	4.5057	1.4242	1.6000
Hyperolius kivuensis	0.0000	0.0385	0.0000	0.0385	0.2307	0.1931	0.1875	0.6113	0.1818	0.1556
Hyperolius lateralis	0.0000	0.0256	0.2500	0.2756	0.4615	1.125	0.8125	2.399	0.7576	0.5555
Hyperolius viridiflavus	0.0222	0.0385	0.0000	0.0607	0.3077	0.4659	1.0000	1.7736	0.3636	0.3333
Kassina senegalensis	0.0000	0.0769	0.0000	0.0769	0.2692	0.3750	0.5625	1.2067	0.3030	0.4222
Phrynobatrachus natalensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0455	0.0000	0.0455	0.0000	0.0000
Xenopus victorianus	0.0444	0.0256	0.0000	0.0700	0.1154	0.0114	0.0000	0.1268	0.0303	0.0222
Ptychadena mascareniensis	0.0000	0.0256	0.0000	0.0256	0.0000	0.0341	0.1250	0.1591	0.0303	0.0222
Amietia angolensis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0114	0.0625	0.0739	0.0000	0.0222
Total	**0.111**	**0.3075**	**0.5000**	**0.9313**	**2.7306**	**4.3523**	**5.0625**	**12.0685**	**3.2423**	**3.6681**

In the case of the overall transect walks performance; the number of species rose rapidly with the initial increase in the number of walks. The number of species then formed a plateau as more walks were added. A small upward movement of the curve follows this indicating that new species were added to the already existing number. Even within the plateau there is a rise and fall in the curve showing changes in the numbers of species being counted. The results are as shown in figure 3.1a-g.

a



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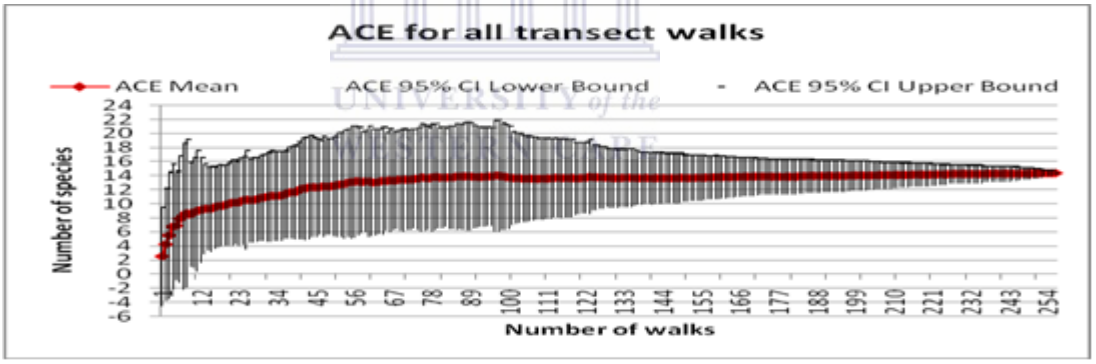

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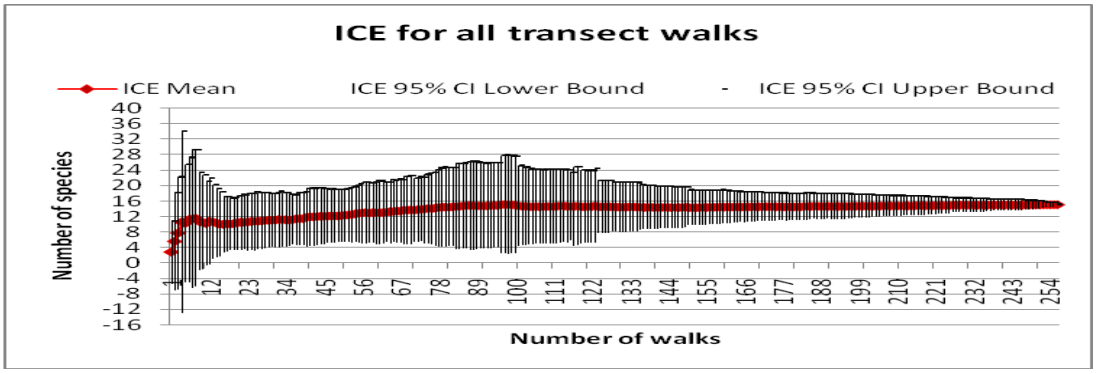

Figure 3.1 a-g. Continued

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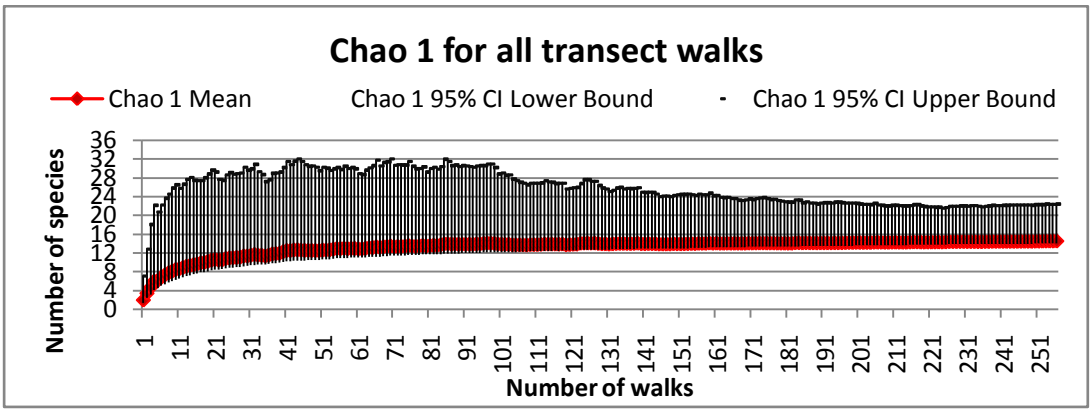

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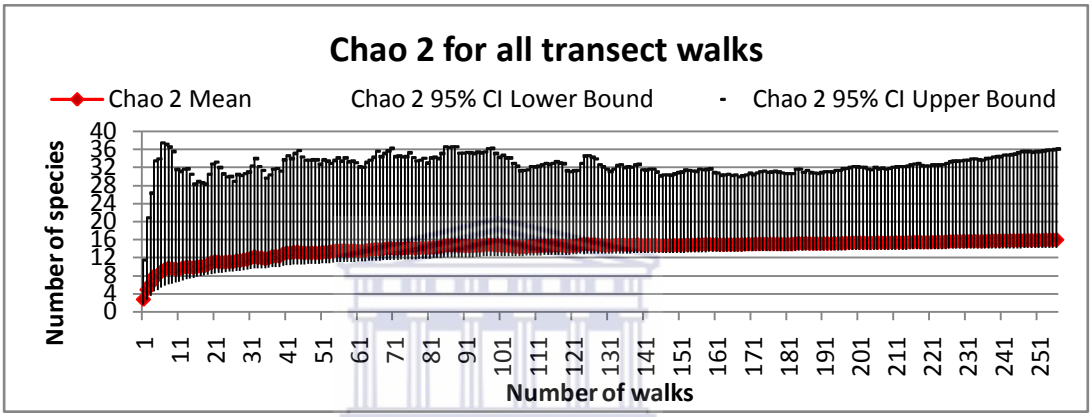

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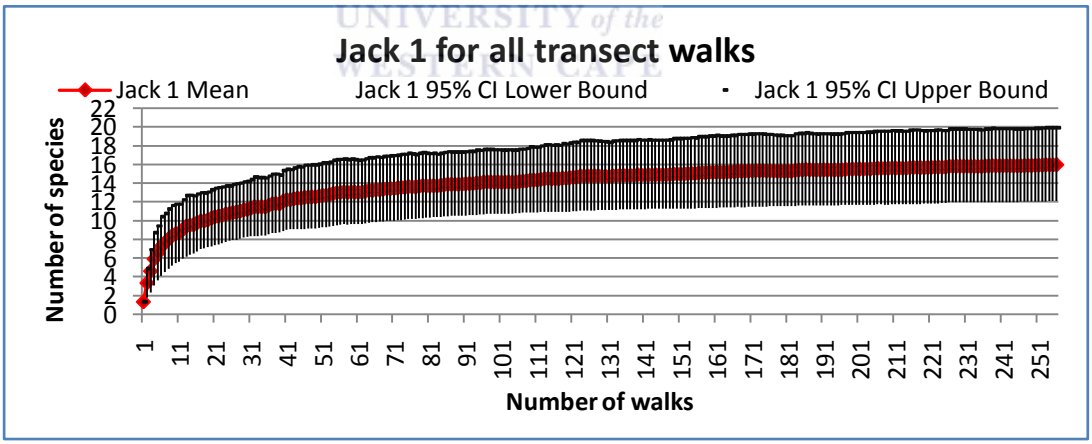

Figure 3.1 a-g. Continued

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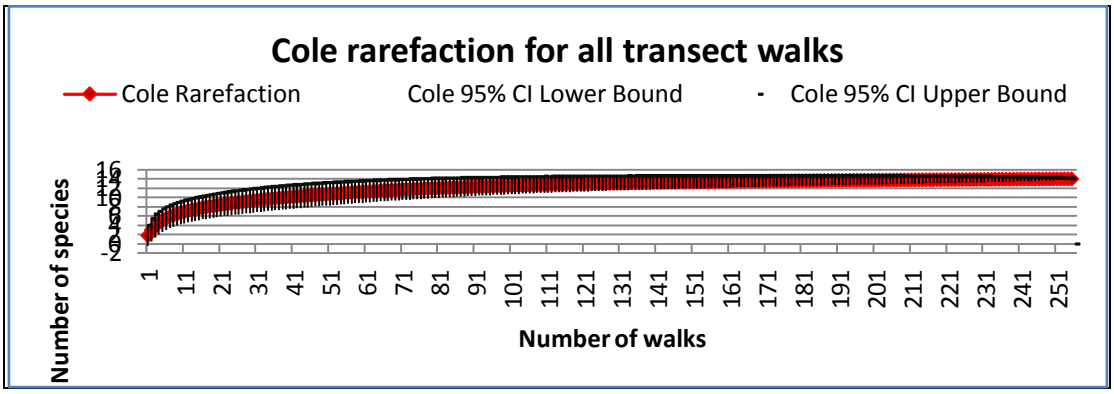
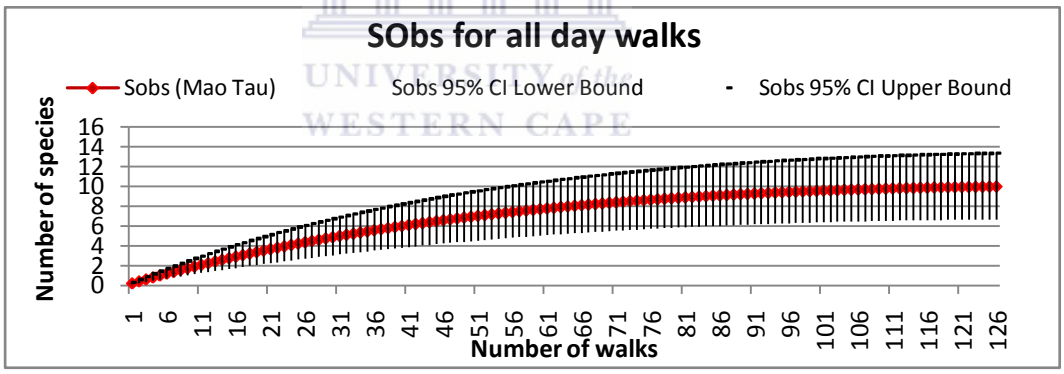


Figure 3.1 a-g. Curves showing different estimators for the overall number of transect walks undertaken

For the overall number of walks during the day, species increase with increased walks, reach a peak and start declining (figure 3.2 a-g). The residues from the upper and lower confidence intervals in the First-order Jacknife are smaller as compared to that of Chao 1 and Chao 2.

a



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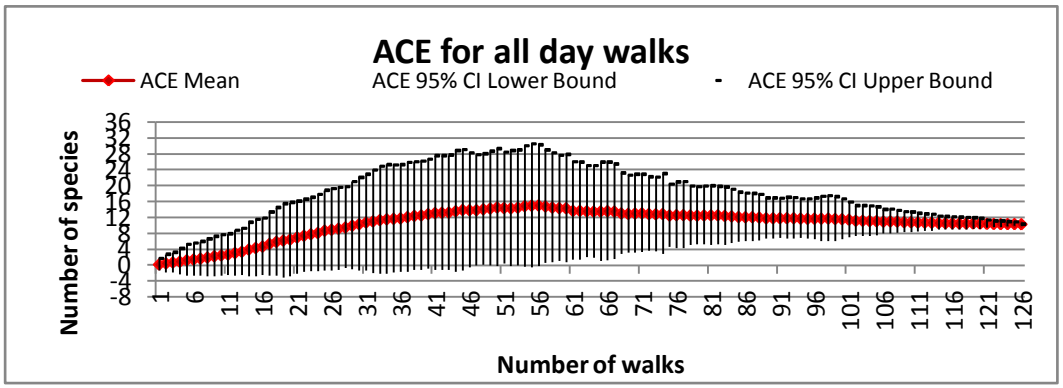

Figure 3.2 a-g. Continued

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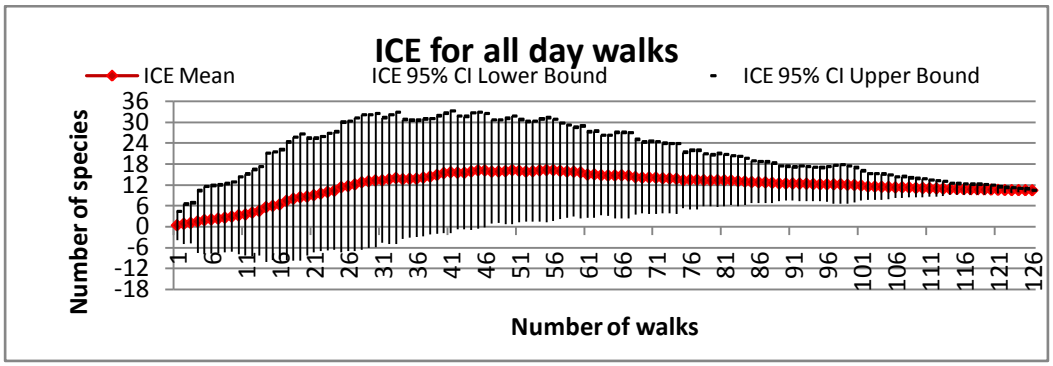

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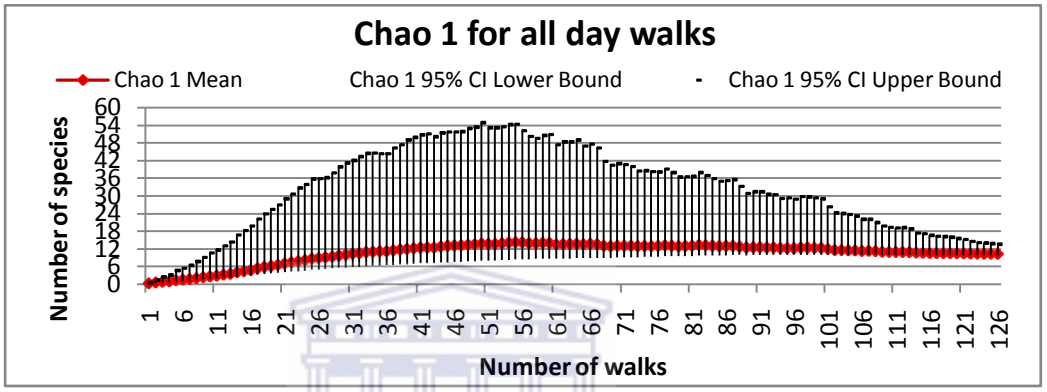

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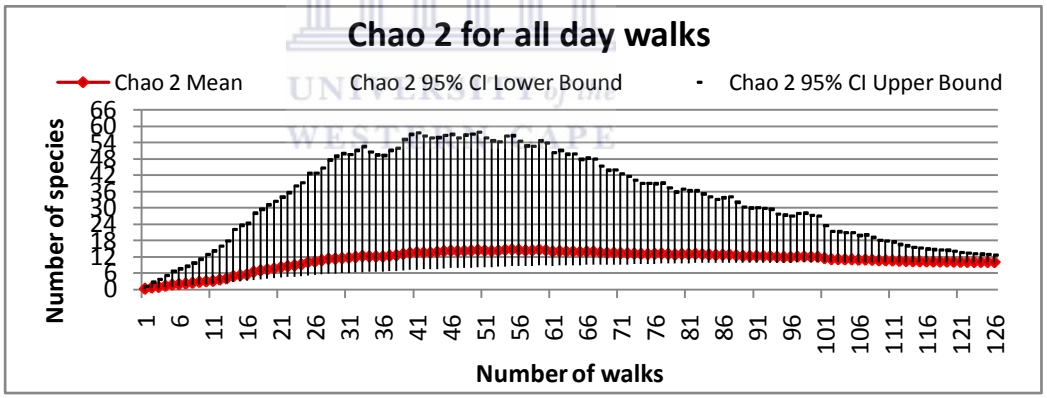

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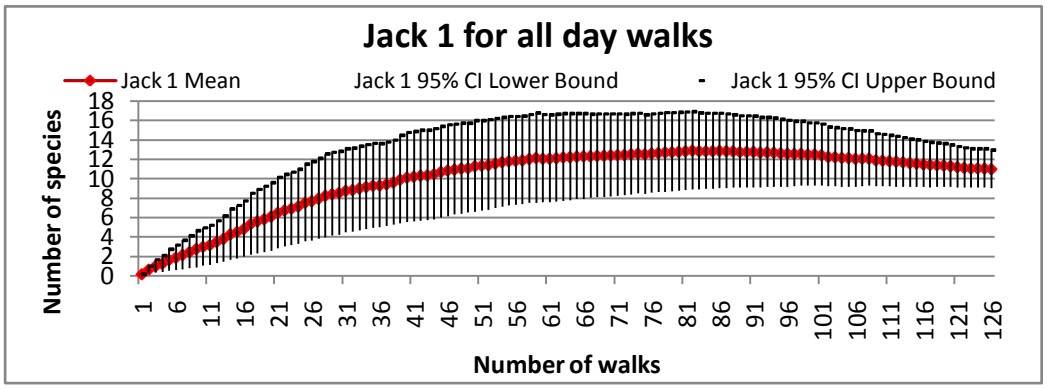

Figure 3.2 a-g. Continued

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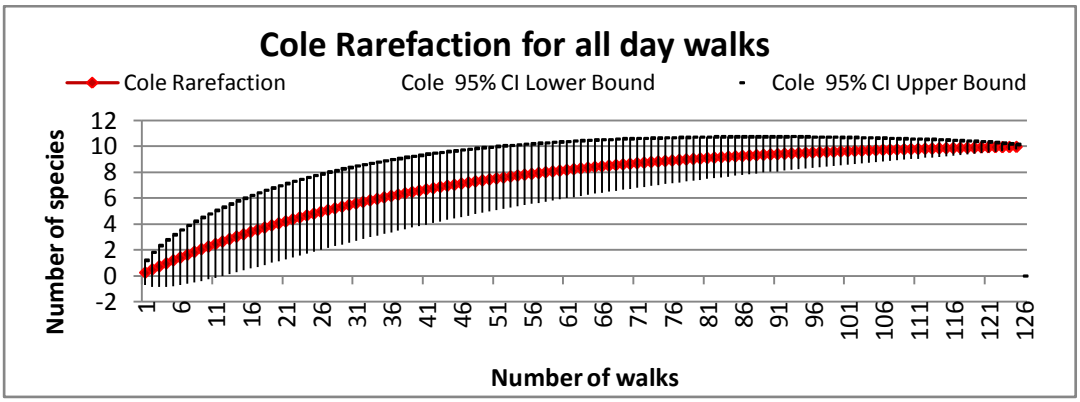
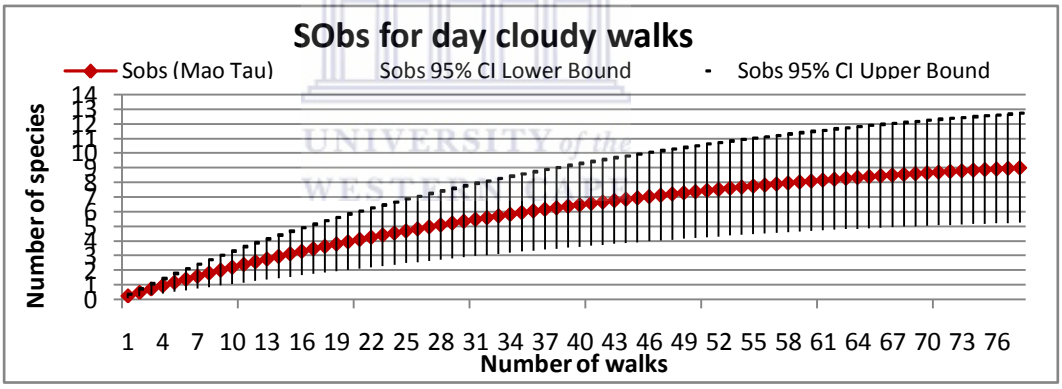


Figure 3.2 a-g. Curves for the various estimators for all the walks during the day

For the day cloudy walks (Figure 3.3 a-g), the numbers keep fluctuating even though increasing in number while there were only two species recorded for the rainy walks during the day.

a



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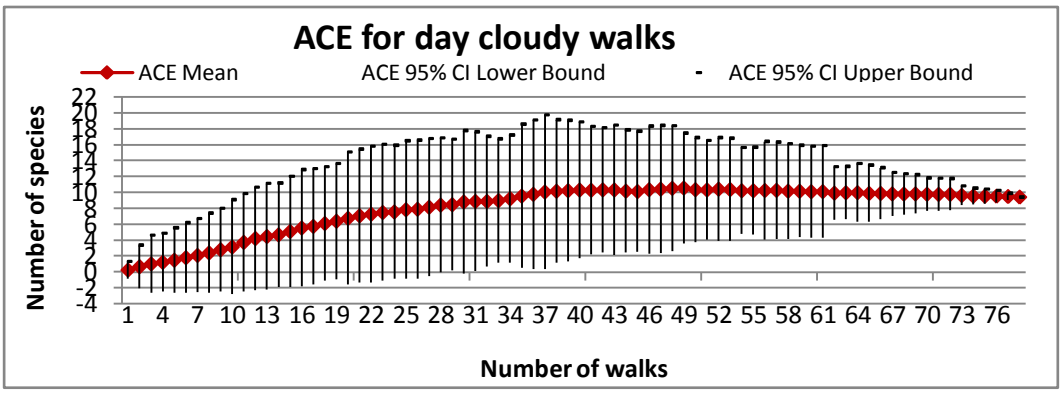

Figure 3.3. a-g. Continued

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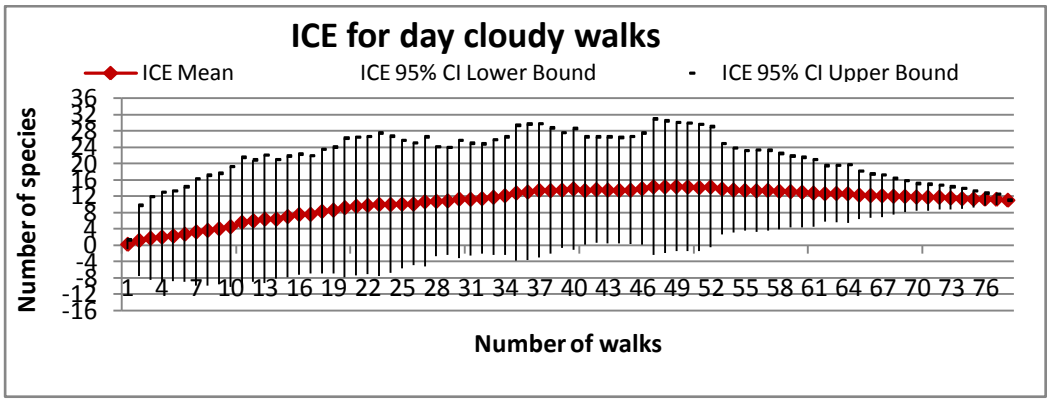

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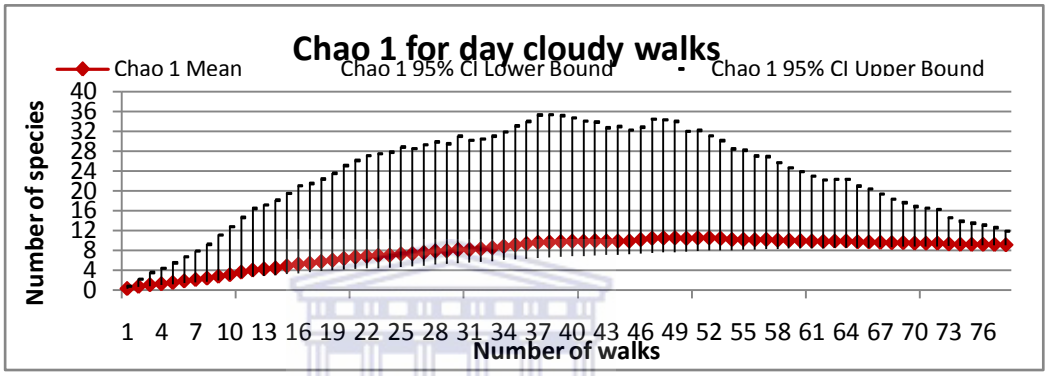

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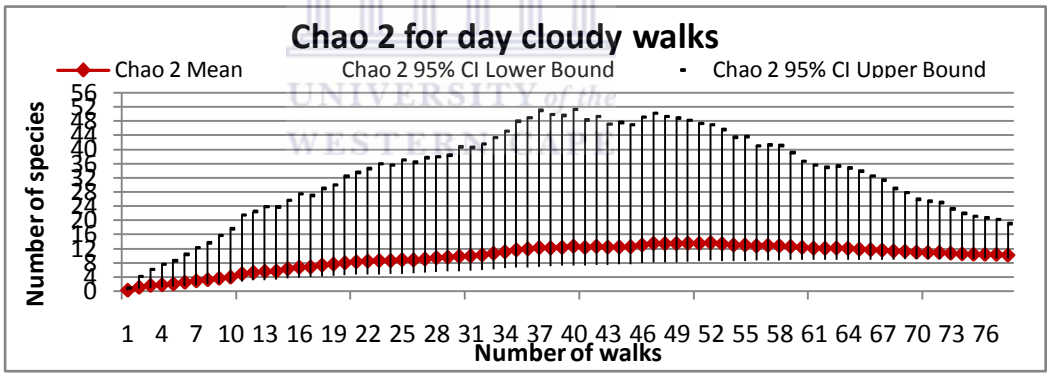

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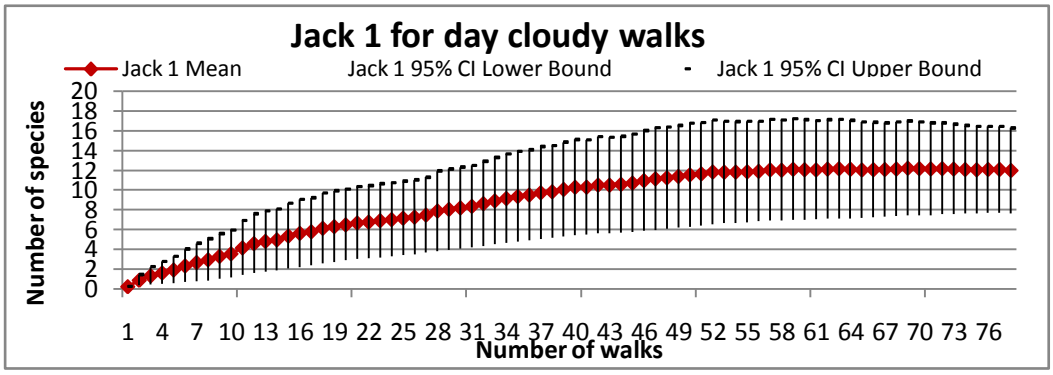


Figure 3.3. a-g. Continued

g

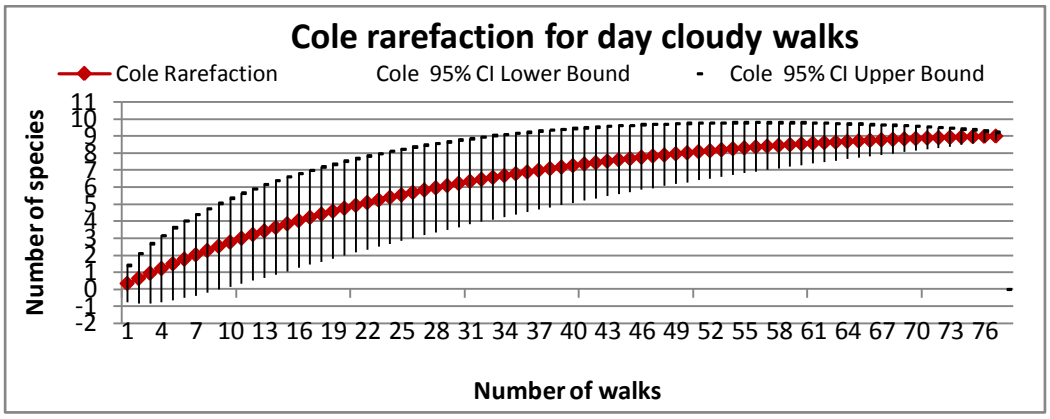
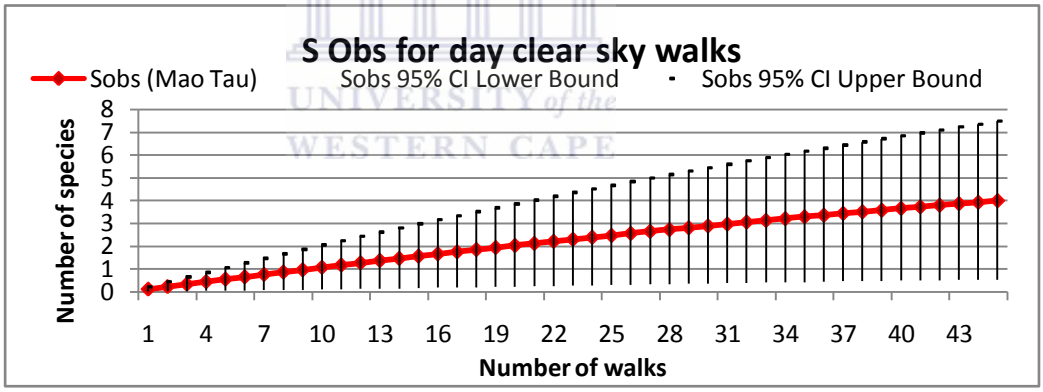


Figure 3.3 a-g. Curves for the various estimators for cloudy walks during the day

In the case of day walks carried out under clear sky, the species kept on increasing considerably without forming a plateau (figure 3.4 a-g).

a



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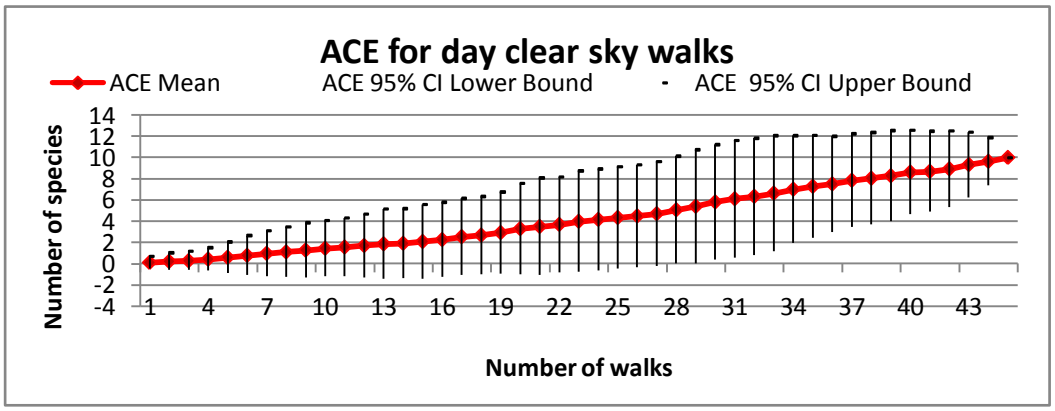

Figure 3.4. a-g. Continued

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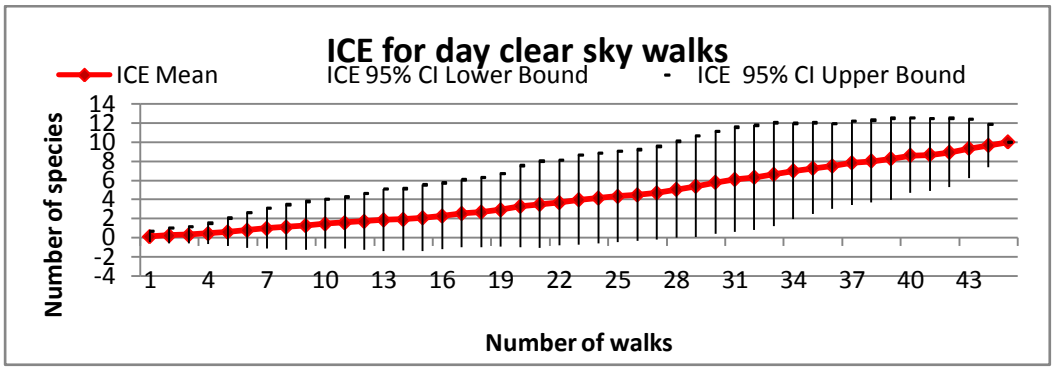

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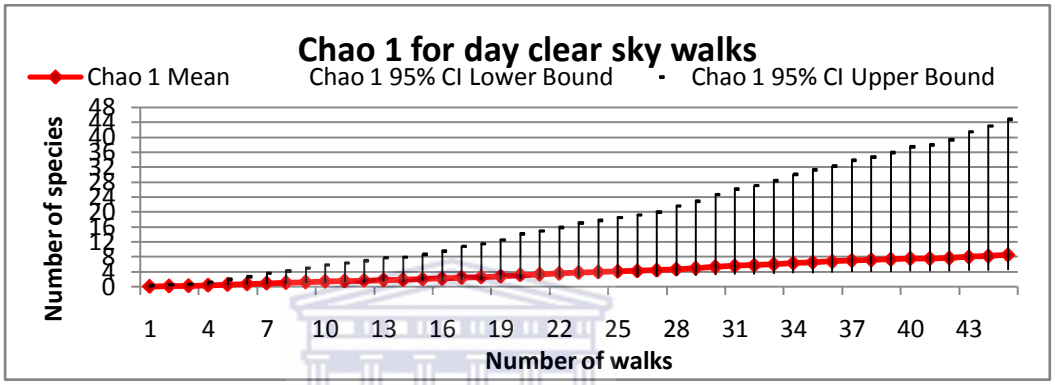

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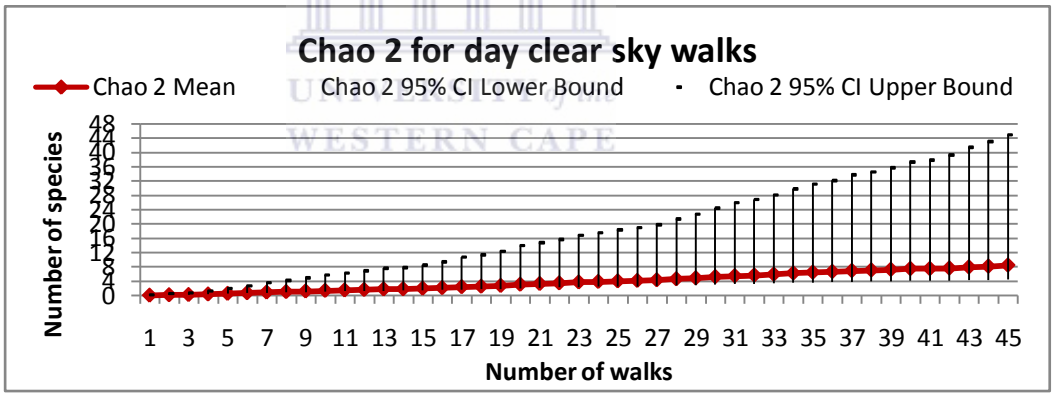

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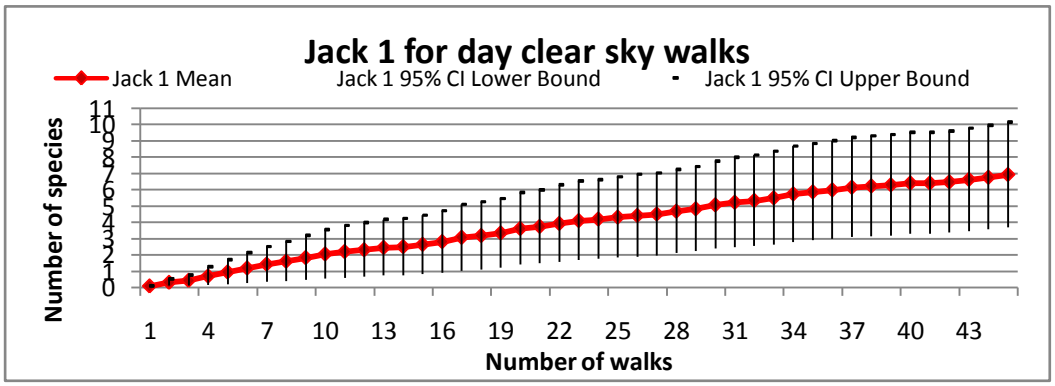


Figure 3.4. a-g. Continued

g

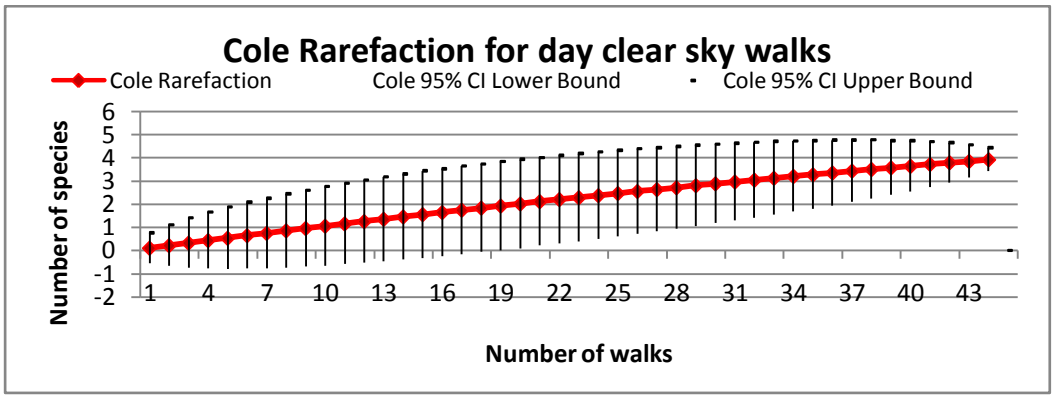
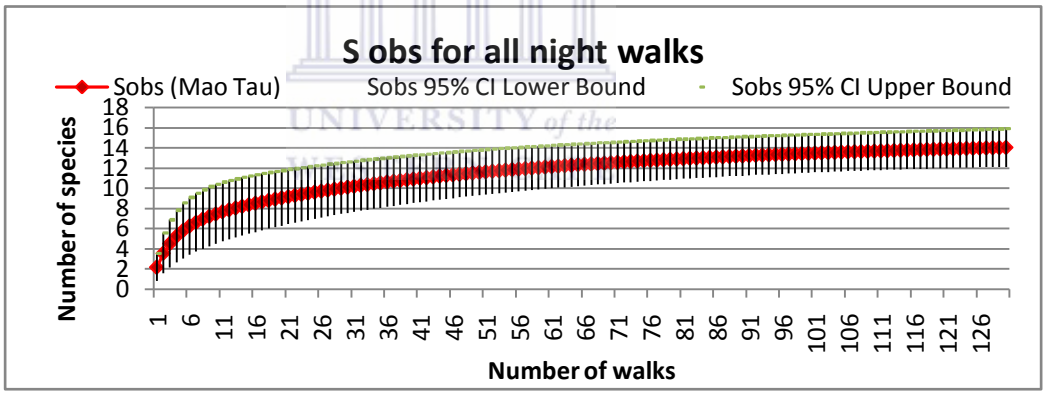


Figure 3.4 a-g. Curves for the various estimators for clear sky walks during the day

In the overall night walks, the species curve did not form a plateau with increase in number of walks (figure 3.5 a-g).

a



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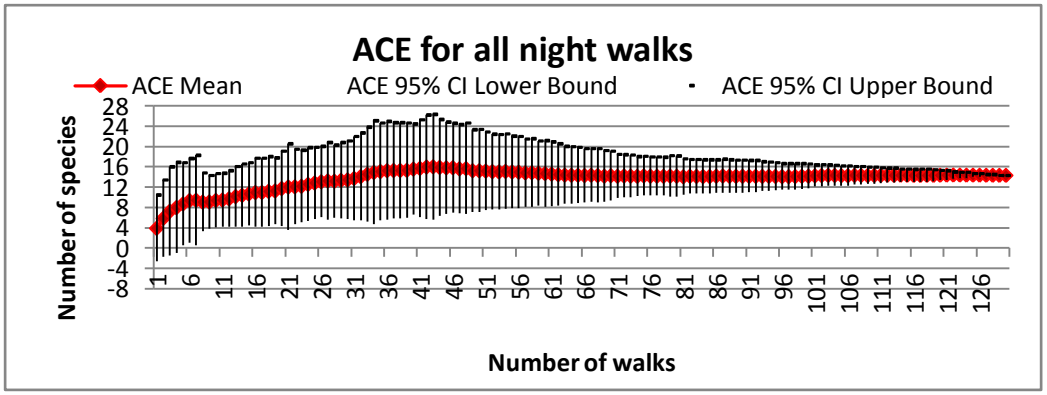
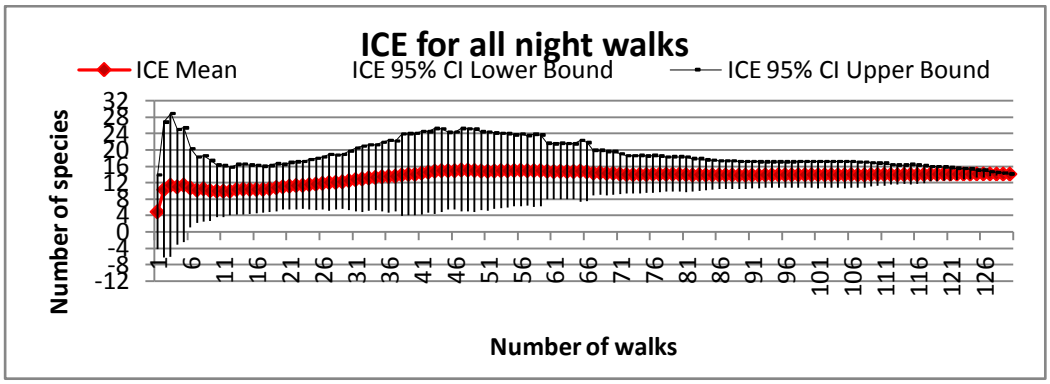


Figure 3.5. a-g. Continued

c



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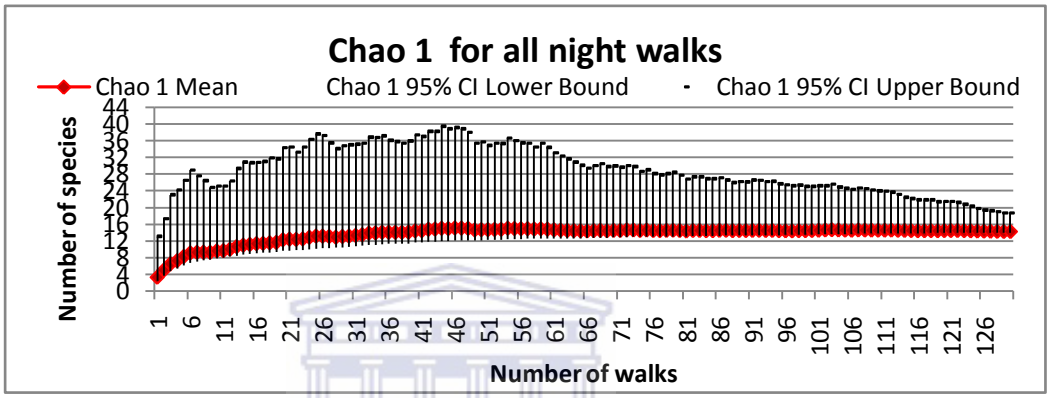

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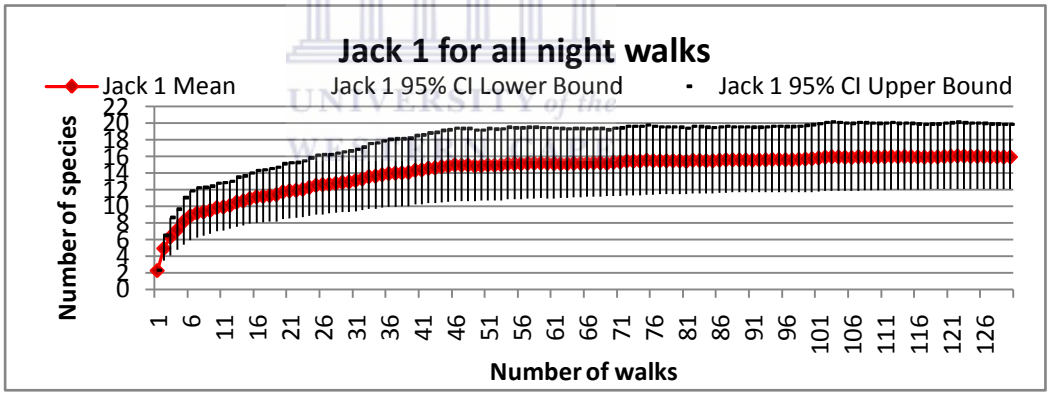

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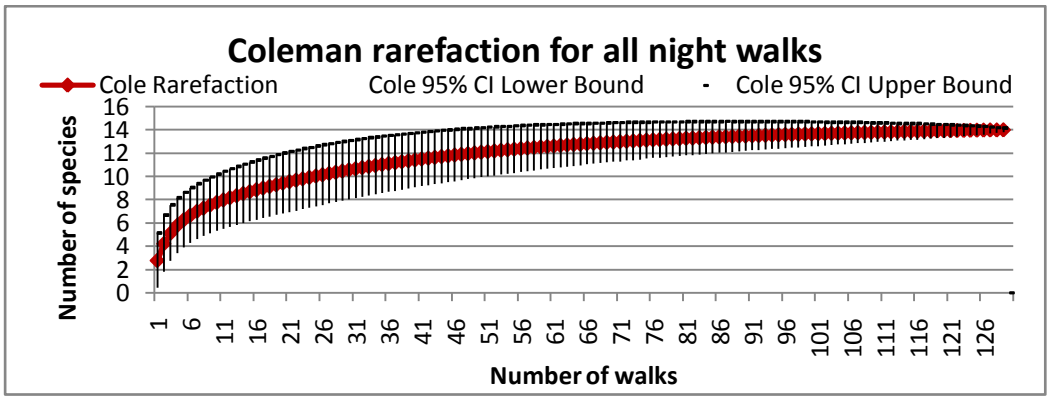
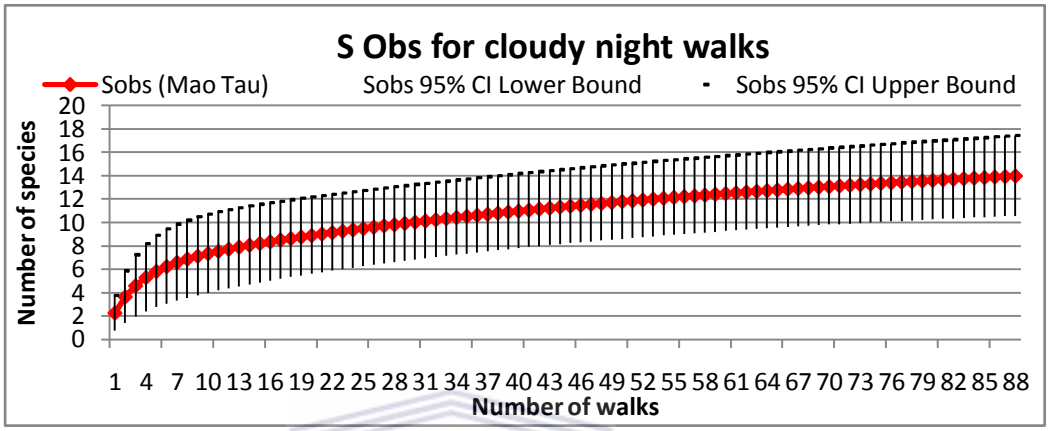


Figure 3.5 a-g. Curves for the various estimators for all the walks at night

The night cloudy(figure 3.6) walks do not form a plateau but keep rising while clear sky walks at night (figure 3.7). only to form a plateau after 14 walks The night walks carried out when it was raining increase initially and then form a plateau after 10 walks (figure 3.8).

a



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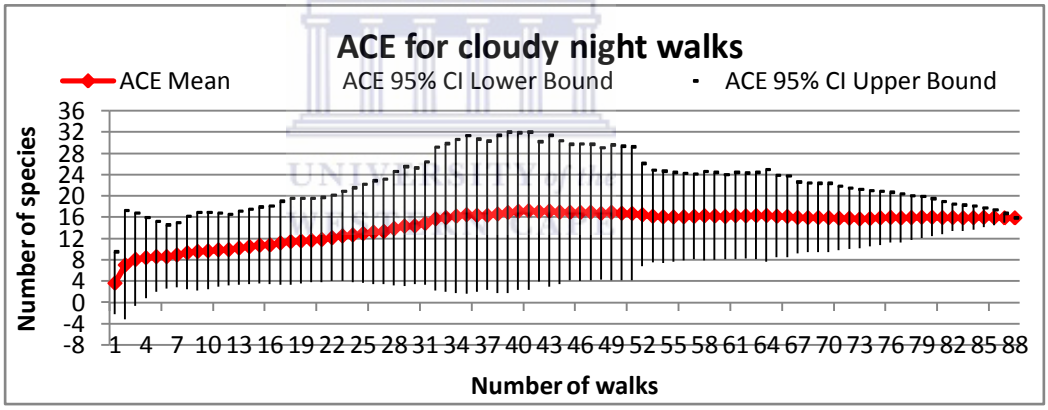

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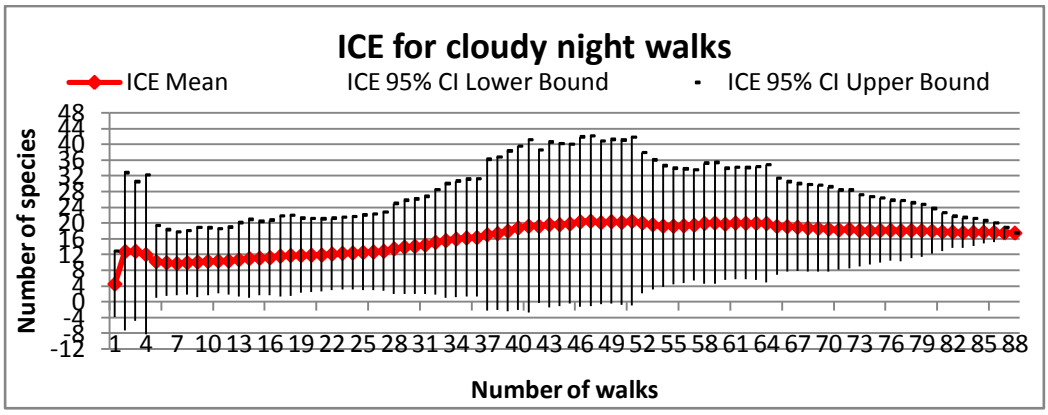

Figure 3.6. a-g. Continued

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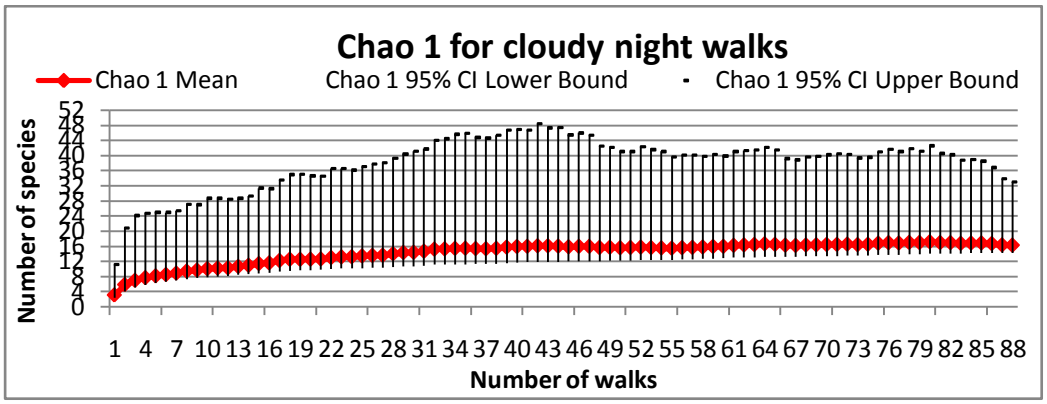

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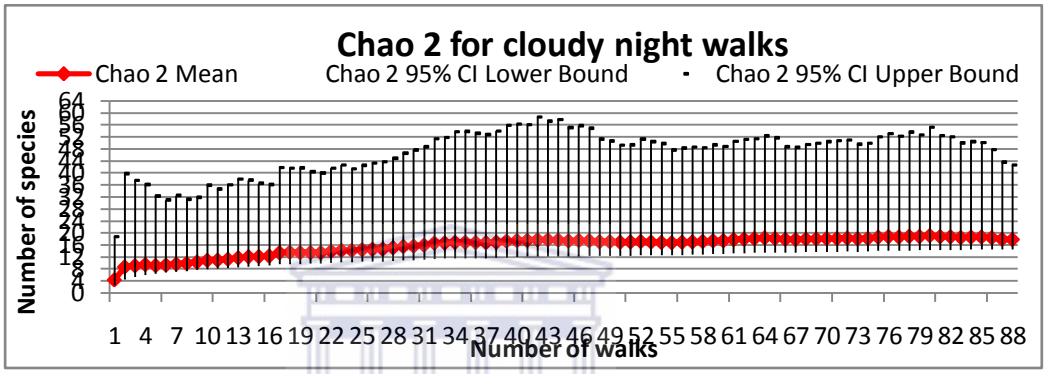

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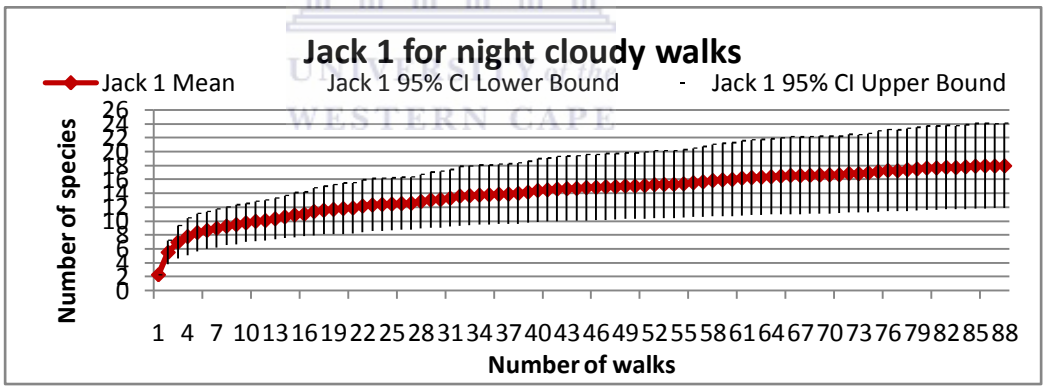

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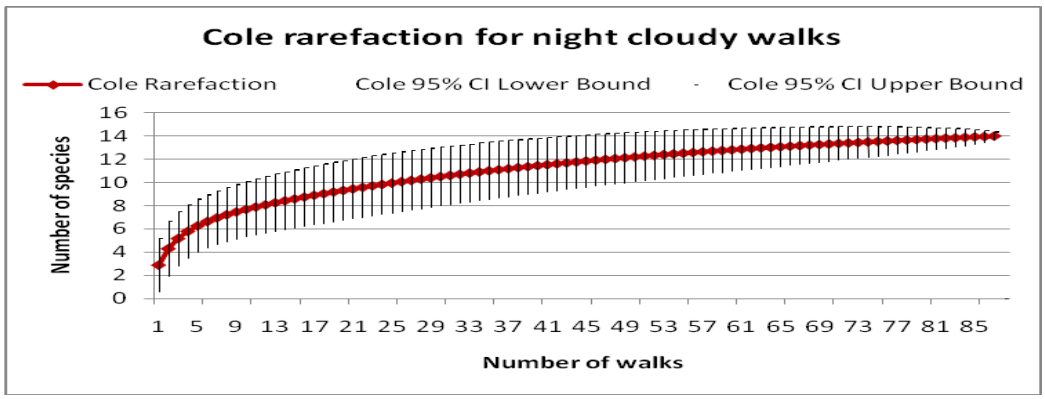
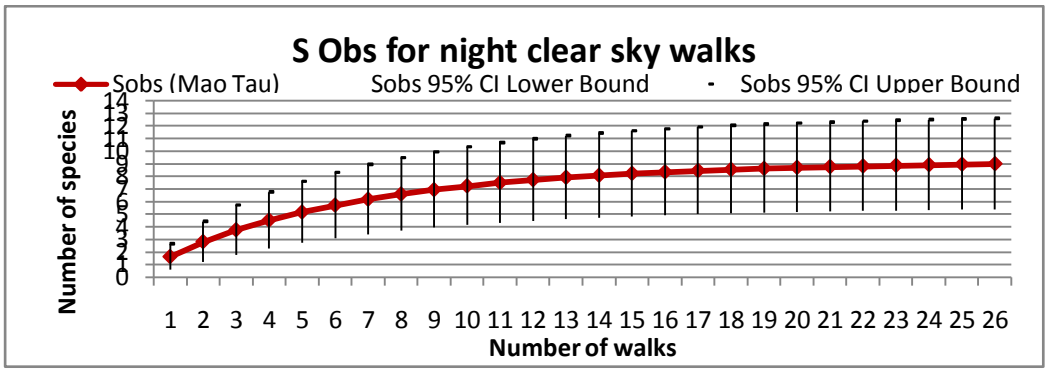


Figure 3.6 a-g. Curves for the various estimators for cloudy walks at night

a



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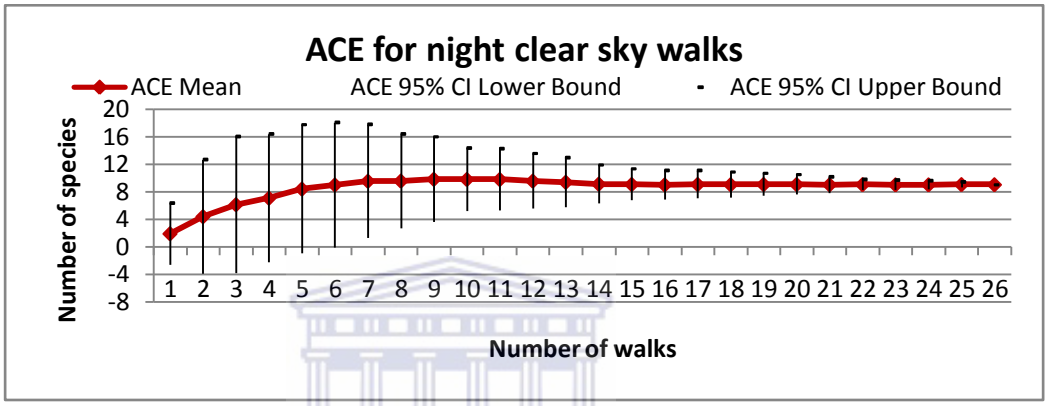

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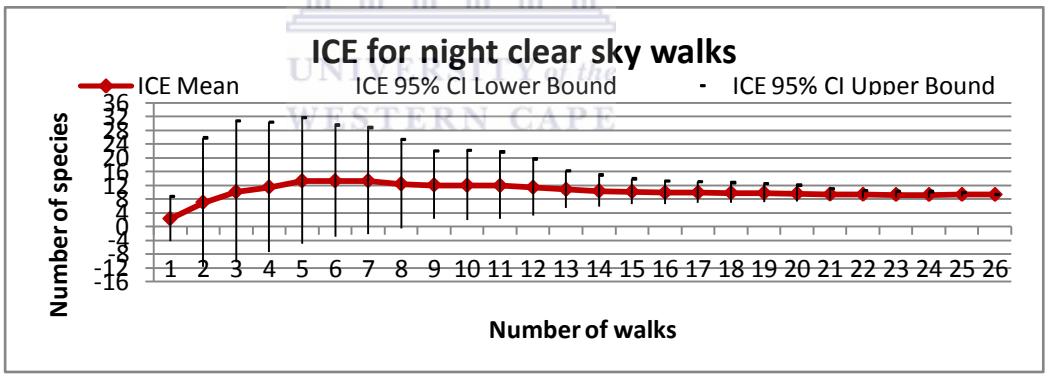

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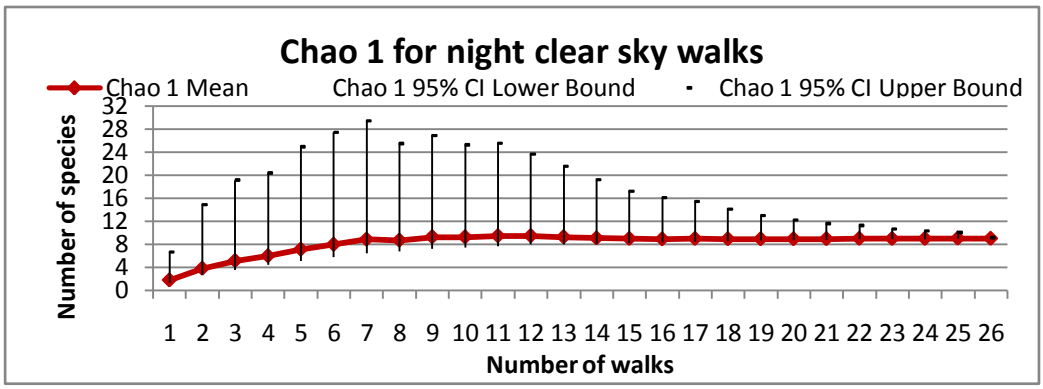
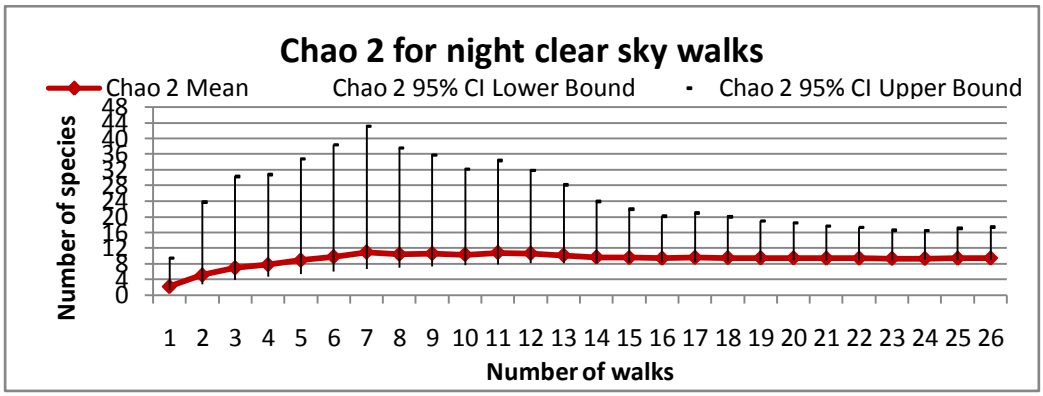


Figure 3.7. a-g. Continued

e



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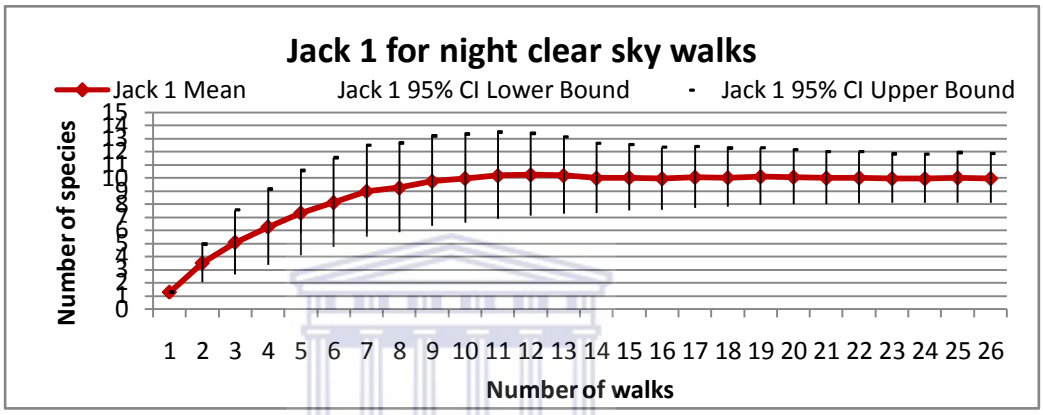

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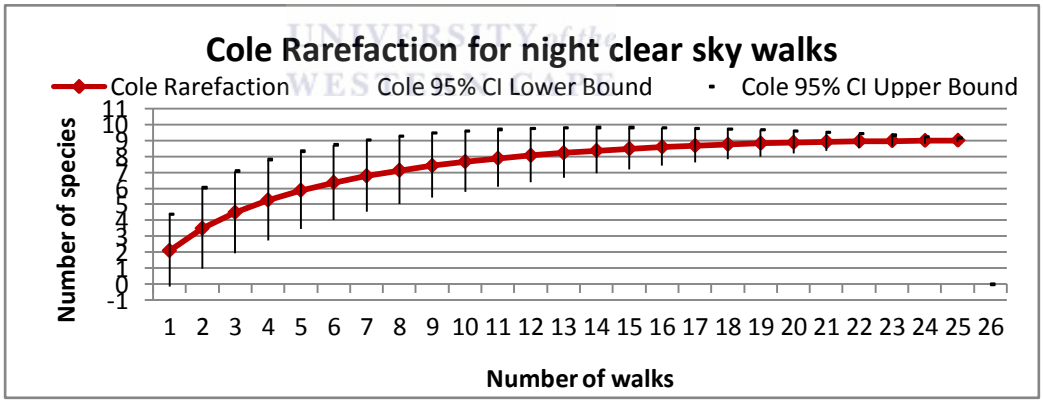
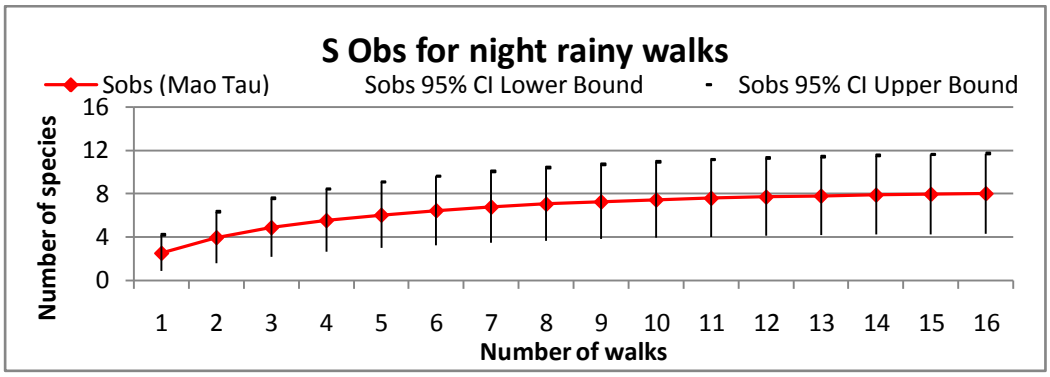


Figure 3.7 a-g. Curves for the various estimators for clear sky walks at night

a



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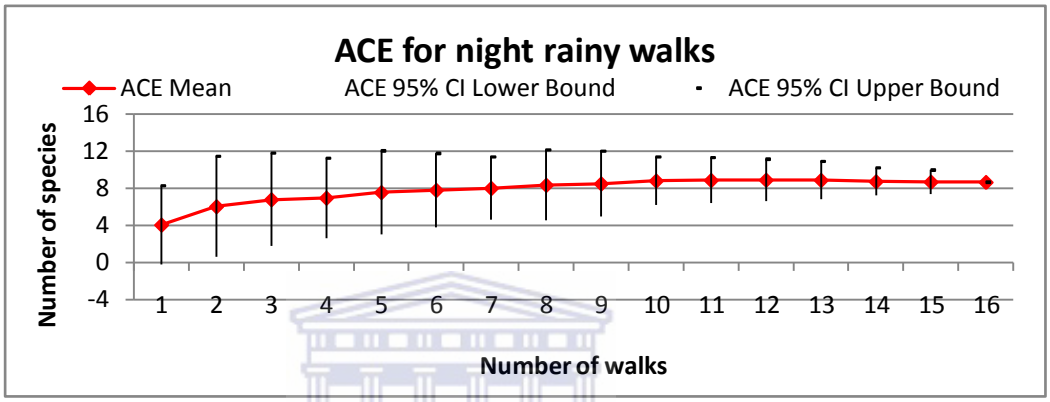

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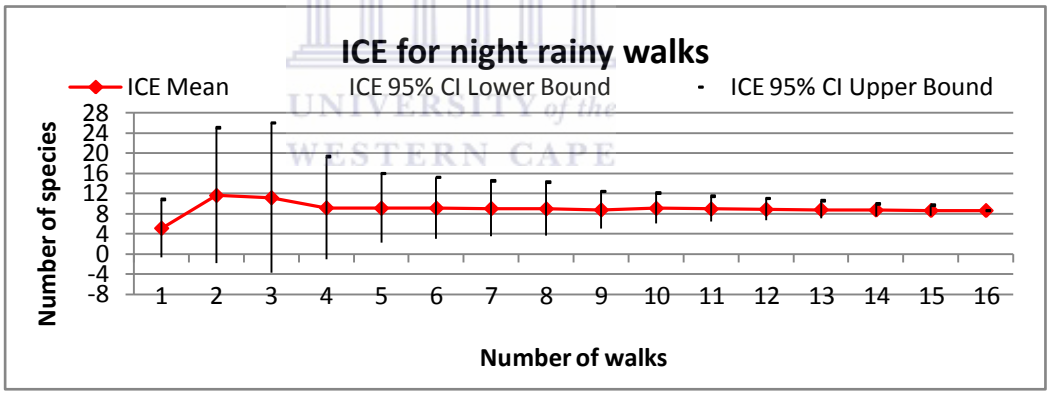


d

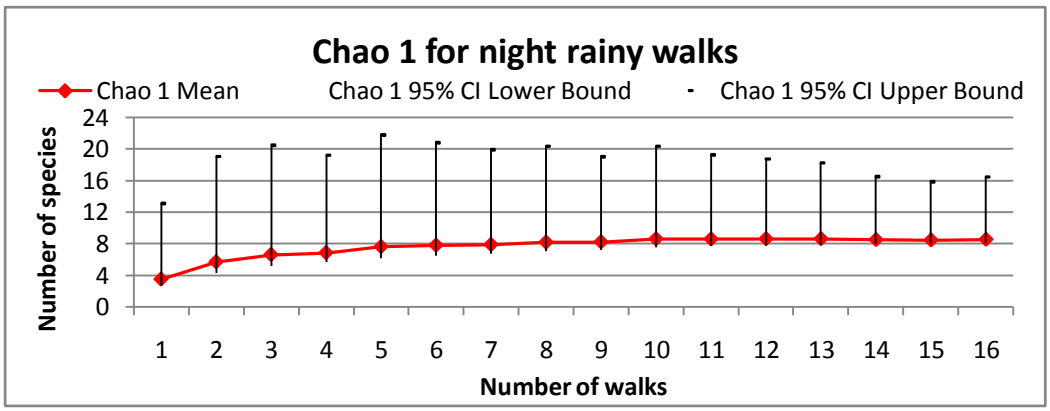
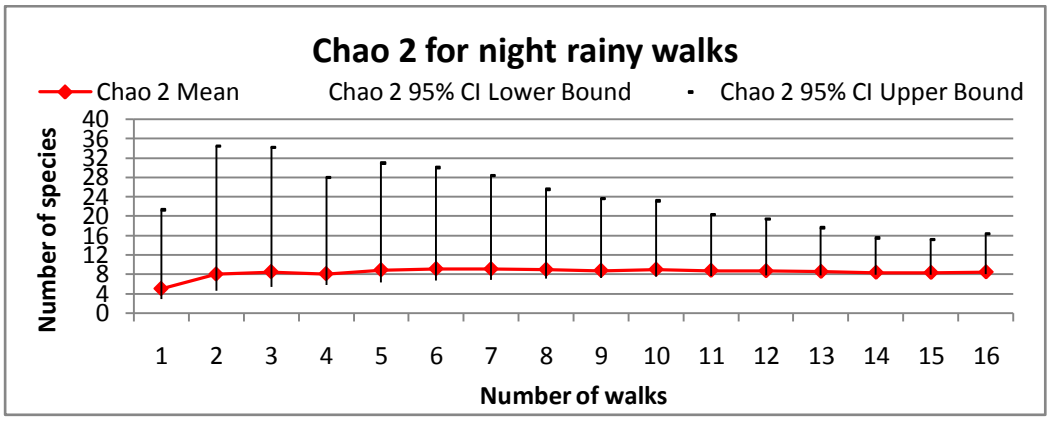


Figure 3.8. a-g. Continued

e



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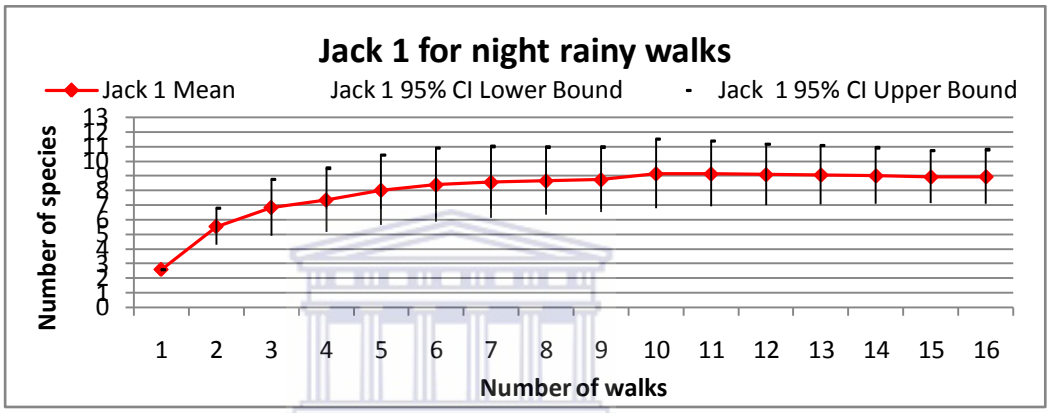

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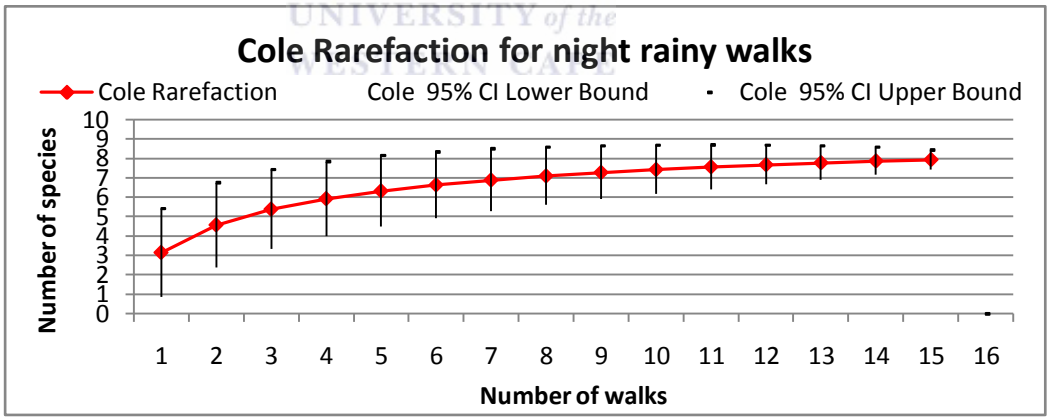

Figure 3.8 a-g. Curves for the various estimators on rainy nights

First-order Jackknife (Jack 1) estimator shows better performance as compared to all other estimators by having the species curves reaching constancy in all the parameters measured.

ICE and ACE followed, with Chao 1 and Chao 2 being the worst performers as shown in figure 3.1.

On the overall number of walks it took 131 walks to sample 12.6 species counted in the transect. During the day, it took 84 walks to sample seven out of the total nine species found. It took 60 walks to sample eight species during the day when cloudy. When it was sunny, it took 39 walks to sample 3.6 of the species.

The total number of species counted at night was 14. It took 73 walks to count 12.6 of the species. A similar number of species was counted when it was cloudy at night, though it took 62 walks to attain 12.6 of these species. Nine species were counted when it was raining and when there was no cloud cover at night. It took 10 and 14 walks for the rainy and clear sky nights respectively to sample 8.1 species.

Table 3.2 Summary of observed and estimated number of frog species of different species richness estimators (ICE, ACE, Chao1, Chao2 and Jack 1) for different weather conditions

Weather conditions	Sobs mean	Sobs s.d.	ACE Mean	ACE s.d.	ICE mean	ICE s.d.	Chao1 mean	Chao1 s.d	Chao2 mean	Chao2 s.d.	Jack1 mean	Jack1 s.d.
Cloudy Day	9	1.91	9.38	0.00	11.01	0.00	9.13	0.44	10.13	1.77	11.96	2.2
Rainy Day	2	1.31	3.00	0	3	2.75	3.00	2.04	2.75	1.57	3.50	0
Clear sky Day	4	1.77	10.00	0	10	0	8.5	7.19	8.5	7.19	6.93	1.65
All Day	10	1.76	10.32	0.25	10.4	0	10.1	0.38	10.17	0.54	10.39	0.99
Cloudy Night	14	1.76	15.84	0.00	17.43	0.00	16.25	3.40	18	5.29	17.95	3.12
Rainy Night	9	1.97	10.91	0.00	10.62	0.00	11	3.74	11	3.74	10.88	1.28
Clear sky Night	9	1.85	9	0.20	9.3	0.00	9.00	0.06	9.5	1.32	9.96	0.96
All Night	14	0.96	14.33	0.01	15.12	0	14.25	0.73	15	1.87	15.98	1.98
All walks	14	1.32	14.34	0.21	15.14	0	14.5	1.32	14.18	3.74	15.99	1.99

4.5 Discussion

Night sampling was the most effective time for conducting this survey. There were 535 specimens counted as compared to 31 during the day. This is because most of the species are crepuscular to nocturnal (Channing & Howell; 2006, Lötters *et al.*, 2007; Stewart 1967; Veith *et al*., 2004). Night time has a higher humidity and has a lower temperature, which frogs need to avoid evaporation from their permeable skins (Bell & Donnelly, 2006). Day walks yielded fewer species, which agrees with the findings of Bell & Donnelly (2006) who found the same in Costa Rica.

Frogs have permeable skins and lose water through evaporation (Duellman & Trueb, 1986) and therefore avoid direct sunlight. This explains the reason why sampling when sunny yielded fewer specimens than when it was cloudy and raining during the day. *Amietophrynus maculatus* and *A. kisolensis* have thick glandular skin that protects them from evaporative water. *Hyperolius viridiflavus* was found near water, while *Xenopus victorianus* is an aquatic species. *Hyperolius cinnamomeoventris* and *H. lateralis* were the only species counted during the day when it was raining.

More species and specimens were counted during the sampling times with clouds than in all the other conditions. Cloudy walks would happen before or after rains resulting in increased number of frogs because of moisture. Rainfall is known to interfere with the acoustic transmission of *Anaxyrus microscaphus* calls and is therefore avoided by this species (Dorcas & Foltz, 1991). This may have led to increased activity after the rains have subsided and therefore no interference. Rainfall could have interfered with the hearing ability of the observer when it was raining resulting in fewer species and specimens counted than when it was cloudy. Clouds reduce the evaporative dehydration caused by increased temperature from the direct sunlight. The frogs therefore do not need to retreat to shaded areas to avoid dehydration, as they would do when temperatures are high (Duellman & Trueb, 1986). During

cloudy weather, light intensities change (Stewart, 1985) and it gets dark earlier than for days with no clouds.

Byrne (2002) found more *Crinia georgiana* mating around the full moon phase. FitzGerald & Bider (1974) found the activity of *Anaxyrus americanus* to be higher around the new moon phase. In this study there were no significant differences between the number of frogs sampled during the new and the full moon phases (p=0.963). Most of the transect is covered by trees, therefore moon light penetration is low. FitzGerald & Bider (1974) notes that the amount of moonlight influences the activity of frogs but the effect will be masked by other factors like rainfall and temperature. In this study, the influence of the moon phases is masked by cloud cover.

More of the arboreal species of the family Hyperoliidae were sampled as compared to terrestrial and aquatic species. They were easier to locate acoustically and visually as compared to other species when calling. *Ptychadena mascareniensis* and *Phrynobatrachus natalensis* have higher visual capability and tend to escape when approached (Veith *et al*., 2004). They also tend to be camouflaged during the day but can be easily spotted using a torch during the night. *Amietophrynus kisolensis* and *A. maculatus* were recorded mainly through SAES and few through SVES due to timidity and their diurnal tendencies.

Xenopus victorianus had equal numbers during the day and at night. They were mainly found in seasonal streams within the forest along the transect even though two specimens were encountered while migrating. The frogs therefore would be sampled during the wet months. The rest of the species except *P. graueri* and *H. albolabris* were recorded in Kakamega forest in the course of the study (Lötters *et al.,* 2006).

Of the 24 species targeted for monitoring only 14 were encountered in the sampling. Only a small portion of Kakamega Forest was studied, whereas the species list covered the whole forest and surrounding areas. *Leptopelis bocagii*, *Phrynobatrachus graueri* and *Hydrophylax albolabris* had been recorded in the forest in previous years (Lötters *et al.*, 2007, Veith *et al*., 2004) but not in the current study.

The number of species in the species richness curves fluctuates mainly due to the effects of the amount of rainfall (refer to Chapter 1). Some of the species are prolonged breeders and others explosive breeders, which leads to them being encountered at different times of the year. In theory the species curves are expected to form a plateau when all the species are sampled in a study area (Magurran, 2004) but this did not happen. This is because of new species being added to the number found and more could be counted if sampling continued (Chao, 2005; Krebs, 1998, and Smith & Van Belle, 1984). In terms of the estimators' performance, First-order Jackknife was the best estimator followed by ACE and ICE. Veith *et al*. (2004) found the same estimator useful in the study of the frogs in Kenya, Indonesia, Ivory Coast and Madagascar.

For this kind of survey, I recommend more than 73 and 84 walks when sampling at night and during the day respectively.

Chapter 4: QUANTIFYING ESTIMATOR BIAS BETWEEN TWO OBSERVERS MONITORING AMPHIBIANS THROUGH TRANSECTS WALKS

4.1 Introduction

Long term amphibian monitoring is necessary as a method to study amphibian decline and extinction (Halliday, 1996). Chapter Two describes the various methods that can be applied in monitoring of amphibians depending on the aims of a study. Transect walks is one of the methods applied in monitoring amphibians and was applied in this study (see Chapter Two). It applies a combination of two sampling methods, SVTS and SAES (Jaeger, 1994; Rödel & Ernst, 2004; Veith, *et al*., 2004). When using acoustic encounters it is possible to identify also morphologically cryptic species through advertisement calls but has the disadvantage of being gender-biased. SVTS on the other hand is not gender biased but has reduced incidence of detection (Rödel & Ernst, 2004; Veith, *et al.*, 2004). SAES provides accurate results for prolonged breeders as compared to the explosive breeders (Zimmerman, 1994) which have a short breeding period.

Transect walks can be carried out by one or more observers. When there is more than one observer there is the probability of detecting an observer error (bias) in the data due to people's different ability to detect specimens (Zimmerman, 1994; Crump & Scott, 1994). Apart from this, species are sometimes difficult to identify, some look alike and have few features that distinguish one from the other (e.g. Carruthers, 2001; Hoffman & Bloun, 2000; Stewart, 1967).

Hyperolius cinnamomeoventris, H. kivuensis and *H. lateralis* are similar in many of their morphological features but each has distinct calls which can be used to distinguish them (Hoffman & Bloun, 2000; Lotters *et al.*, 2004; Schiøtz, 1999). The family Ptychadenidae has

species which are morphologically distinguished by inner thigh markings (Carruthers, 2001; Stewart, 1967). This is difficult to detect especially during walks if the specimens are not caught as they are timid and escape as they are approached (Veith, *et al*., 2004).

Not only are anuran morphologies similar but also their advertisement calls. Rödel & Ernst (2002) observed that both the morphology and advertisement calls of *Phrynobatrachus alticola* and *P. guineensis* are similar. On the other hand the calls of *Amietophrynus asmarae* and *A. regularis* were found to be similar when heard in the field but differed when their spectrograms were compared (Tandy *et al.,* 1985). *Hyperolius lateralis* and *H. kivuensis* have similar calls and morphology so that some species are impossible to identify (Schiøtz, 1999). This can lead to the wrong identification of specimens.

Another source of observer bias is the amount of time that is spent in looking for specimens on the ground and those on vegetation (Crump & Scott, 1994). SAES though being strong in identification of species (Rödel & Ernst, 2004) requires observers having proper training and to be able to identify calls properly. This is because calls may sound different at different temperatures (Duellman & Trueb, 1986). These biases can be minimised through practice, adequate training and randomising sampling if undertaken by different individuals (Jaeger, 1994).

There are limited data on errors caused by observers when undertaking amphibian sound surveys with Zimmerman (1994) recommending the application of information from acoustic avian studies. Bart (1985) found three observer errors; undercounting, over counting and wrong identification of species. He notes that observers can record species other than the actual ones present in the study site while others prefer to record some species as compared to others. Bart & Schoultz (1984) concluded that there is no method for adjusting data between observers but gives ways to improve efficiency. However, they suggest that the error when there are not many

species in a particular area should not exceed 25% and 35% when there is a high density of species.

Observer efficiency is affected by type of species present, number of species present and other noises present in a particular area (Bart & Schoultz, 1984; Zimmerman, 1994). With more experience there is reduced bias but differences between observers cannot be completely overcome (Bart & Schoultz, 1984; McLaren & Gadman, 1999). Zimmerman (1994) notes that observer errors are more likely for species with calls above 4 kHz and those with high calling rates where there are large choruses.

4.2 Aims

In this chapter, I wanted to know whether there are significant differences between two observers' data when using the same sampling methods under similar environmental conditions.

4.3 Methods

Two observers (Victor Wasonga, observer A, and Beryl Akoth, observer B), after being trained in a similar way, carried out transect walks as described in Chapter Two, but alternately between January and June 2006. Part of the training for the two observers was to distinguish calls of species with similar calls, for example: *Hyperolius cinnamomeoventris, H. lateralis* and *H. kivuensis*. Data was collected and sorted as described Chapters Two and Three. The number of species and specimens sampled by each individual are presented in Table 4.2.

Clear sky and cloudy night walks had data for both observers and therefore could be compared as recommended by Zimmerman (1994). To eliminate the bias arising from the differences in number of walks a sampling index was calculated. It was expressed as a ratio of the number of specimens sampled and the number of walks (Table 4.3). The data was tested for normality and equal variance but did not fulfill the assumptions. A non-parametric equivalent of one way

ANOVA (Kruskal-Wallis) was computed between the sampling index for the two observers for the cloudy and clear sky walks at night conditions using SPSS (Version 14.0).

4.4 Results

A total of 48 walks were carried out shared equally between day and night, and between the two observers.

Table 4.1. The number of transect walks undertaken under different weather conditions for the two observers

Weather conditions	Observer A	Observer B
Rainy (Day)	0	0
Cloudy (Day)	6	7
Clear sky(Day)	6	5
Rainy (Night)	2	2
Cloudy (Night)	6	7
Clear sky (Night)	4	3

All specimens were recorded through SAES, with the exception of two *Hyperolius viridiflavus* of which one was female. A total of eight species were counted during the walks,both observers counting seven species each. All species overlapped except two, since observer A encountered *Amietophrynus maculatus* while observer B *Hyperolius cinnamomeoventris* apart from the other six common species as shown in Appendix 6.7. *H. viridiflavus*, *H. lateralis* and *Kassina senegalensis* were the most sampled species accounting for 75.8% of the total specimens. Both observers sampled more *K. senegalensis* than all other specimens.

A total of 92 specimens were sampled; with only four counted during the day. Observer A recorded 58 specimens in total, 53 at night and four during the day while observer B sampled a total of 34 specimens all during night sampling. Cloudy walks at night accounted for most specimens sampled.

Only clear sky and cloudy walks at night had comparable data between the two observers (Appendix 6.7) which was expressed as a ratio between the number of specimen and the number of walks as in Table 4.2

Table 4.2. Sampling index for clear sky walks and cloudy walks at night for the two observers

Species	Clear sky (Night)		Cloudy (Night)	
Observers	OB A	OB B	OB A	OB B
Hyperolius viridiflavus	0.667	0	1.667	1.286
Hyperolius kivuensis	0.333	1	0.5	0.571
Hyperolius lateralis	1.667	0	1.667	1
Hyperolius cinnamomeoventris	0	0	0	0.143
Kassina senegalensis	0.333	0.5	1.833	1
Afixalus. quadrivittatus	0.667	0	0.833	0.286
Xenopus victorianus	0	0	0	0.143
Amietophrynus maculatus	0.333	0	0	0

There was no significant difference between the two observers for the data collected in the cloudy walks and clear sky walks at night (Kruskal Wallis ANOVA H_3=-0.145, P = 0.986).

4.5 Discussion

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There was no observer bias in the data collected by the two observers in the species and specimens counted. This study demonstrates the need for thorough training of observers before embarking on a monitoring exercise. This especially applies when there is more than one observer involved in sampling. The procedure in ornithological monitoring studies involving two observers is first to have a pilot study before undertaking the main study. Retraining of the observers is done when there are significant differences in the data collected (Githiomi Pers. Comm., 2006).

There is limited data is available for this type of study to make comparisons. However from this study the two observers demonstrate that they can effectively continue with the monitoring without major variations in their data. Both observers were able to distinguish *Hyperolius lateralis* and *H. kivuensis* which according to Hoffman & Bloun (2000), Lotters *et al.*, (2004) and Schiøtz, (1999) are not easy to distinguish.

I recommend more transect walks to compare data under different conditions and seasons. I also recommend adding the time factor as a measurement to know the amount of time spent in sampling (Crump & Scott, 1994), in order to better understand the effect of observer bias in monitoring amphibians.

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6. Appendices

Appendix 6.1. List of voucher specimens from which calls were recorded

Species name	Specimen voucher number
Hyperolius kivuenisis	NMK A/3953
Hyperolius lateralis	No voucher specimen
Hyperolius viridiflavus	ZFMK 77426
Hyperolius acuticeps	NMK A/3922/1-2
Hyperolius cinnamomeoventris	ZFMK 77431-432
Afrixalus osorioi	NMK A/3927
Afrixalus quadrivittatus	NMK A/3933/2
Leptopelis mackayi	ZMFK 83304-305

Appendix 6.2. Specimens counted in different vegetation segments

Species	Primary forest	Secondary forest	Swampy forest	Swampy grassland	Road
Leptopelis mackayi	0	4	41	2	0
Amietophrynus kisolensis	0	2	0	0	2
Amietophrynus maculatus	1	5	0	0	2
Afrixalus quadrivittatus	1	0	3	0	0
Hyperolius acuticeps	0	0	0	1	0
Hyperolius cinnamomeoventris	12	20	14	146	8
Hyperolius kivuensis	1	6	10	10	2
Hyperolius lateralis	11	20	9	87	0
Hyperolius viridiflavus	7	30	8	20	4
Kassina senegalensis	16	8	8	0	23
Phrynobatrachus natalensis	0	0	0	0	4
Xenopus victorianus	1	1	6	0	0
Ptychadena mascareniensis	0	0	0	7	0
Amietia angolensis	0	0	0	2	0
Total	**50**	**96**	**99**	**279**	**45**

Appendix 6.3. Specimens counted in each vegetation segment during normal rainfall (under 200 mm)

Species	Primary forest	Secondary forest	Swampy forest	Swampy grassland	Road
Leptopelis mackayi	0	3	28	0	0
Amietophrynus kisolensis	0	0	0	0	1
Amietophrynus maculatus	0	1	0	0	2
Afrixalus quadrivittatus	1	0	1	0	0
Hyperolius acuticeps	0	0	0	0	0
Hyperolius cinnamomeoventris	3	10	8	80	2
Hyperolius kivuensis	1	3	6	3	2
Hyperolius lateralis	2	6	1	15	0
Hyperolius viridiflavus	3	20	3	14	3
Kassina senegalensis	7	2	3	0	13
Phrynobatrachus natalensis	0	0	0	0	0
Xenopus victorianus	0	1	2	0	0
Ptychadena mascareniensis	0	0	0	6	0
Amietia angolensis	0	0	0	0	0

Appendix 6.4. Specimens counted in each vegetation segment during high rainfall (over 200 mm)

Species	Primary forest	Secondary forest	Swampy forest	Swampy grassland	Road
Leptopelis mackayi	0	1	13	2	0
Amietophrynus kisolensis	0	2	0	0	1
Amietophrynus maculatus	1	4	0	0	0
Afrixalus quadrivittatus	0	0	2	0	0
Hyperolius acuticeps	0	0	1	0	0
Hyperolius cinnamomeoventris	9	10	6	66	6
Hyperolius kivuensis	0	3	4	7	0
Hyperolius lateralis	9	14	8	72	0
Hyperolius viridiflavus	4	10	5	6	1
Kassina senegalensis	9	6	5	0	10
Phrynobatrachus natalensis	0	0	0	0	4
Xenopus victorianus	1	0	4	0	0
Ptychadena mascareniensis	0	0	0	1	0
Amietia angolensis	0	0	0	2	0

Appendix 6.5. Formulae for the different estimators used

S_{obs}

$$S_{Obs} = \sum_{j-1}^{H} s_j$$

ACE

$$S_{ace} = S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}} + \gamma_{ace}^2$$

ICE

$$S_{ice} = S_{freq} + \frac{S_{rare}}{C_{ice}} + \frac{Q_1}{C_{ice}} + \gamma_{ice}^2$$

Chao 1

$$S_{Chao1} = S_{Obs} + \frac{F_1^2}{2F_2}$$

Chao 2

$$S_{Chao2} = S_{Obs} + \frac{Q_1^2}{2Q_2}$$

Jack Knife 1

$$S_{Jack1} = S_{Obs} + Q_1 \left\{\frac{m-1}{m}\right\}$$

Variables definition

J=Number of samples

Appendix 6.5

s_j = Number of species found in j samples

H=Total samples

S_{obs}- Total number of species observed

S_{rare}- Total number of rare species

S_{abund}- Total number of abundant species

m= Total number of species

Q_1/Q_2= Number of species that occur in a certain number samples (Q_1 is the frequency of unique and Q_2 is the frequency of duplicates)

F_1 /F_2= Number of species that have exactly a certain number of number of individuals (F_1 Frequency for unique and F_2 is the frequency of duplicates)

γ_{ace}^2 = Estimated coefficient of frequency variation of the rare species

γ_{ice}^2 = Estimated coefficient of frequency variation of the infrequent species

C_{ace} = Sample abundance coverage estimator

C_{ice} = Sample incidence coverage estimator

Appendix 6.5.

Appendix 6.6. Total specimens for each species under different weather conditions and moon phases on the transect (D=day, N= Night)

Species name	Clear sky (D)	Cloudy (D)	Rainy (D)	Clear sky (N)	Cloudy (N)	Rainy (N)	New Moon (N)	Full moon (N)
Leptopelis mackayi	0	2	0	3	37	5	3	19
Amietophrynus kisoloensis	1	1	0	0	2	0	1	0
Amietophrynus maculatus	1	0	0	2	4	1	0	4
Afrixalus quadrivittatus	0	0	0	2	2	0	1	0
Hyperolius acuticeps	0	0	0	0	1	0	0	0
Hyperolius cinnamomeoventris	0	4	1	26	138	31	47	72
Hyperolius kivuensis	0	3	0	6	17	3	6	7
Hyperolius lateralis	0	2	1	12	99	13	25	25
Hyperolius viridiflavus	1	3	0	8	41	16	12	15
Kassina senegalensis	0	6	0	7	33	9	10	19
Phrynobatrachus natalensis	0	0	0	0	4	0	0	0
Xenopus victorianus	2	2	0	3	1	0	1	1
Ptychadena mascareniensis	0	2	0	0	3	2	1	1
Amietia angolensis	0	0	0	0	1	1	0	1
Total	**5**	**25**	**2**	**69**	**383**	**81**	**107**	**164**

Appendix 6.7. Summary of the number of specimens sampled by the two observers under different weather conditions except rainy periods (OB= Observer)

Species	Clear sky (day)		Cloudy (day)		Clear sky (Night)		Cloudy (Night)		Rainy (Night)	
Observers	OB A	OB B	OB A	OB B	OB A	OB B	OB A	OB B	OB A	OB B
Hyperolius viridiflavus	0	0	0	0	2	0	10	9	0	0
Hyperolius kivuensis	0	0	0	0	1	2	3	4	0	0
Hyperolius lateralis	1	0	0	0	5	0	10	7	0	0
Hyperolius cinnamomeoventris	0	0	0	0	0	0	0	1	0	0
Kassina senegalensis	1	0	2	0	1	1	11	7	2	0
Afixalus. quadrivittatus	0	0	0	0	2	0	5	2	0	0
Xenopus victorianus	0	0	0	0	0	0	0	1	1	0
Amietophrynus maculatus	0	0	0	0	1	0	0	0	0	0