The effect of sampling on the measurement of small mammal diversity; species abundance and species richness

Bachelor Thesis
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Abstract

The research describes the effect of sampling on the measurement of biodiversity, expressed with the variables species abundance and richness, in general and specific for a small mammal research done by Chimbo Foundation (2014). The effects are highlighted in the light of four major sampling aspects; Sampling location, marking technique, species' specific characteristics and trap disturbances. The use of a transect and placing of traps at different heights positively influence the measured diversity. The marking technique biases the measured species diversity when marks are not maintained during the whole research period. Causing a more flattened species accumulation curve and an overestimation of species abundance. Information about species' specific characteristics (lifecycle, home range and dispersal patterns) is incomplete. The impact of this aspect on the measurement of biodiversity is therefore hard to determine and is mostly based on speculations and assumptions. The best known trap disturbances are caused by larger animals and ants. These disturbances includes dismantling of traps, removal of bait and/or mutilating/killing of captured animals, which all lead to a lower measured species diversity. Overall it can be concluded that sampling always influence measured diversity, either positive or negative depending on the choices made, to a certain extent. Chimbo measured the species richness, which was done very optimal. However, a change which can result in a huge improvement is the use of another marking technique, like ear tags. This causes maintenance of marks during the whole research period, which leads to an unbiased species accumulation curve and the possibility to measure species abundance. This can be very worthy in the broader aim of Chimbo Foundation; The set up of a protection program for the Boé region, Guinea-Bissau.

Introduction

Biodiversity is the rich variety of life on Earth. Biodiversity can more specifically be defined as all levels of natural variation from the molecular and genetic levels to species level (Huston & Huston, 1994). Beyond the species level biodiversity includes variations in nature up to patterns in the landscape level (Huston & Huston, 1994). In this study the term biodiversity will only refer to variations on species level.

Ecosystems provide a variety of goods and services to humanity. An ecological system is composed of different species of micro-organisms, plants, fungi and animals. Humans change the composition of biological communities by activities that increase species invasions and extinctions rates (Hooper et al., 2005). Species' functional characteristics strongly influence ecosystem properties such as productivity, carbon storage, hydrology, and nutrient cycling (Hooper et al., 2005). These properties are not only affected by the abundant species, but also by the relatively rare species in the ecosystem (Hooper et al., 2005). Therefore, when setting up ecosystem protection programs, knowledge of the present biodiversity in the ecosystem is necessary.

The biodiversity of micro-organisms, plants, fungi and animals in an ecosystem can be enormous. Therefore, in practice only one specific group can be investigated at a time. Since there is this limit, it is useful to choose a group which can give information about the whole ecosystem. Animals and more specific small mammals is a group which can accomplish this, caused by their important role in the food chain, in seed, spores and propagules dispersal and in soil preparation (Coetzee, 1975; Moura, Grelle, & Bergallo, 2008; Pearce & Venier, 2005). Therewith, the use of small mammal diversity as an bio-indicator of ecosystem 'integrity' is suggested in recent studies (Avenant, 2011; Carey & Harrington, 2001; Pearce & Venier, 2005). This indication is a result of; the dominance of generalists in the community at low ecological integrity, the number of specialist increasing towards ecological climax and specific species acting as indicators during the succession process (Avenant, 2011).

To determine small mammal diversity, samples needs to be collected. Samples of small mammals are usually collected by capturing the animals with the use of live traps, instead of direct sights. Studies which focuses on small mammal diversity are for example; Datiko & Bekele, (2013), Decher, Kilpatrick, & Bahian, (2001), Makundi, Massawe, Mulungu, & Katakweba, (2010), and the small mammal research of Chimbo Foundation[1], (2014). However, the way of capturing influence the quality of the sample and thereby the measured diversity. This has led to the following research question: Which effect does sampling have on measuring small mammal diversity? This question will be answered in the light of four aspects of capturing, each influencing the measured diversity in a certain way. First, the sampling location, this is the basis when obtaining data. Second, the marking technique, marks prevent double counting, which can lead to an overestimation of the amount of individuals of a species. Third, species' specific characteristics, these influence captures directly. Fourth, trap disturbances, there are other animals than the target species which can be attracted by the live traps. These animals can cause disturbances in the collected data. Together, they give the basic information of the most important effects of sampling on measuring small mammal diversity, applicable to any small mammal research. This basis can be expanded with more species and location specific variables, like trap response, trap type and bait type when a small research is set up. Each of the four aspects mentioned above corresponds to a chapter. Every chapter starts with a general introduction. Next, the possible influence of the chapters specific aspect on measuring diversity will be discussed. Also the influence of decisions which can be made with respect to the aspect will be mentioned. After this, there will be explained which choices were made during the small mammal research of Chimbo. Finally, the effect of these specific choices on the measured small mammal diversity will be discussed.

Before the four chapters, there will be a brief introduction in how biodiversity can be measured and in the general set up of the small mammal research of Chimbo. After the four

[1]Chimbo Foundation: A Dutch foundation with the objective to maintain and where necessary restore the chimpanzee population in West Africa and the natural environment in which they live.

chapters there will follow a discussion, including the general conclusions. Finally recommendations specific for the small mammal research of Chimbo will be given.

The measurement of biodiversity

As mentioned earlier, biodiversity will be seen as variations on species level. To give a value to diversity, there is a need of specific variables which can express this. The first and most fundamental variable is species richness (Peet, 1974). Species richness reflects the number of species, arising from counting, present at a given location (Ricotta, 2005). However, this is a very crude estimation of community structure. To express diversity in a more complete way, the distribution of species relative abundances has to be taken into account, also known as the evenness component (Ricotta, 2005; Tuomisto, 2010). The essence of this evenness component can be seen when the diversity of a community with five equally abundant species and a community with the same five species, but with one species comprising 95% of the individuals are compared (Peet, 1974). In both cases the species richness is the same. However, when two individuals are selected at random from each community, they are much more likely to represent different species in the first case than in the second case (Peet, 1974). This last point reflects the difference in evenness. Because of this, both communities should not be considered to have the same diversity. Therefore, diversity indices which quantitatively measure the combination of both, species richness and evenness, can be useful to give a value of diversity. An increase in species number and/or in species evenness, increases the value of a diversity index. The most common diversity indices are the Shannon index and the Simpson index (Ricotta, 2005). Indices differ in their sensitivity for the presence of rare species (Magurran, 2004). Therefore, indices have to be chosen with respect to the aim of the research (Makundi et al., 2010).

Small mammal research of Chimbo

The small mammal research of Chimbo has the aim to give information about the diversity of small terrestrial mammals in the Boé, Guinea Bissau (figure 1). This survey started at 6-10-2013 and will continue for one year in total. Data of the five months, of dry season, will be used in this report. This survey takes place at five different research locations. The habitats of these locations varies from high grassland savanna to a small gallery forest with a canopy height of approximately ten meter. Ten pairs of live traps, each consisting of a Sherman and a Heslinga live trap, are placed at every location. Besides the live traps, five pitfalls are placed beneath a drift fence of approximately 50 meter. Specimens and photographs of captured individuals are collected for identification. The individuals are released after marking. The collected data must lead to an indication of the small mammal diversity in the Boé. Diversity is expressed in the number of species, also known as species richness, because this way of measuring diversity is relatively easy to perform. The goodness of sampling is visualized with a species accumulation curve. In this curve species accumulation is plotted against specimens captured. When a species accumulation curve reaches a plateau, it can be said that a community is well sampled (Bâ, Kane, Gauthier, & Granjon, 2013; Magurran, 2004). More detailed information of the materials and methods of this research can be found in my research report (attachment 1).

Figure 1: The Boé region. (Goedmakers & Wit, 2014)

The effect of sampling location; trap spacing and trap height

In many biodiversity studies live traps are used to capture small mammals (Datiko & Bekele, 2013; Decher et al., 2001; Makundi et al., 2010). Therefore, sampling locations for the live traps have to be chosen. The choice of sampling locations can affect the measured diversity, caused by roughly two location factors; trap spacing and trap height (Pearson & Ruggiero, 2003; E. M. Vieira & Monteiro-Filho, 2003). Both factors will be discussed in this chapter. Starting with the effect of trap spacing, concerning the positioning of traps on the horizontal level. Followed by the effect of trap height, concerning the positioning on the vertical level.

Trap spacing

Trap spacing concerns the positioning of traps on the horizontal level. There are roughly two spatial designs for live traps; in a grid or in a transect. The effectiveness in sampling diversity of both designs will be compared. Starting with a theoretical comparison, followed by a comparison in practice.

A grid and a transect have different geometries. This results, in a different effective trapping area, with the same amount of traps. Since a trap samples not only the exact spot where it is located, but in addition also samples an adjacent area. This can be schematically seen as a (black) unit, which is surrounded by (grey) units (figure 2). In this way a transect of 25 traps will sample 65% more surface than a grid of 25 traps (Pearson & Ruggiero, 2003). Therefore, the transect design will result in more captures and more species than a grid design. Besides, transects may sample more unique microhabitats by crossing a broader area, which can also increase the number of captured species (Pearson & Ruggiero, 2003).

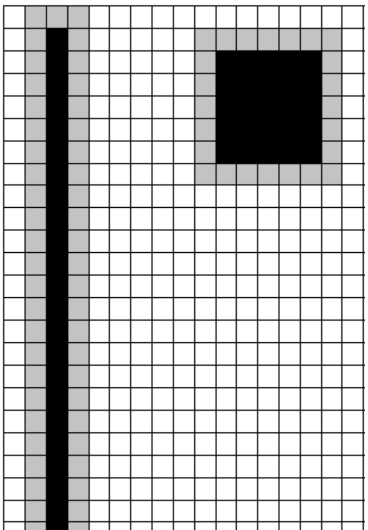


Figure 2: Schematic figure showing an effective trapping area sampled by a transect and grid arrangement

Practical comparisons, however, led to conflicting results for a long time. Some studies showed that transect designs were more suitable for sampling the area than grid designs, but there are also studies showing the opposite (Pearson & Ruggiero, 2003). This conflict is probably the result of the way of comparison. Most studies compared results of studies already done. Therefore, not only the spatial design differed in these comparisons but also some other factors like trap distance (Pearson & Ruggiero, 2003).

The effect of trap distance was investigated in 1988 by Read *et al*. in the eastern foothills of the Brindabella Range, Australia. When trap distance increased, number of captures in the research area declined in a linear way (figure 3). This is not surprising, because in fact trapping density declines. More interesting is that increasing the distance between traps in a grid, decreased capture number faster compared to a transect (figure 3). Therefore, a grid configuration is more sensitive to trap distance.

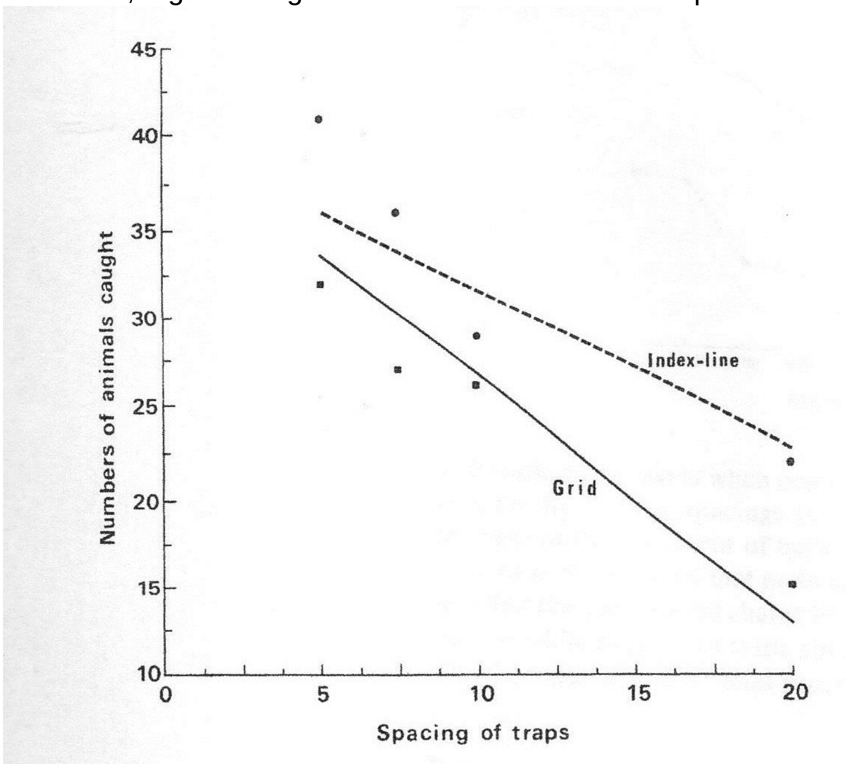


Figure 3: Trap number is plotted against trap distance (meter) for a grid and a transect configuration. Steepness indicates sensitivity for trap distance. (Read, Malafant, & Myers, 1988)

The reliability of both transect and grid configuration with the use of four trapping distances (respectively 5;7,5;10;20) was also checked (Read et al., 1988). The reliability was determined by comparing the found diversity with the expected diversity. This expected biodiversity was determined with four different indices (Simpson, Pielou, Equitability and Brillouin diversity indices). Where a transect was reliable at 5;7,5;10 meters a grid was only reliable by a distance of 5 meters (Read et al., 1988).

According to the effects of trap distance, comparisons of grid and transect studies can only be trusted when the same trap distance is used. Therefore Pearson & Ruggiero, (2003), set up a new study in west central Montana, USA, with the sole aim to compare the results of a grid and a transect design. Therefore, only the design differed, so trap type and trap distance were kept the same. The results were clear, a transect design leads to more captures, more individuals and more species (Pearson & Ruggiero, 2003).

It can be concluded that, theoretical and practical, a transect design leads to the capture of more species and more individuals than a grid. Therefore, a transect is the best design for a diversity study. Keep in mind that these comparisons were made with the sole focus of sampling diversity as well as possible. When other aims are taken into account, like the analysis of movement patterns and home ranges, a grid can be a better design (Pearson & Ruggiero, 2003). This is caused by its compact arrangement, which makes individuals traceable.

Trap spacing in the small mammal research of Chimbo and its effect on measured diversity

In the small mammal research of Chimbo live traps are placed in a transect design with a trap distance of approximately five meter. Both, the design and the trap distance, are optimal for a diversity study according to the literature described above. Therefore, trap spacing had the least possible impact on the measured diversity.

Trap height

When trap spacing is chosen, traps must be placed at a certain height in the vegetation. A trap can be either placed on the ground or at specific heights in trees. Several studies showed a clear vertical stratification of small mammals in tropical forests (Grelle, 2003; Hannibal & Caceres, 2010; Lambert, Malcolm, & Zimmerman, 2005; E. M. Vieira & Monteiro-Filho, 2003). Trap heights can be divided roughly into three categories, ground, understory and canopy. The height positioning of understory traps is approximately 1,7 meter and of canopy traps approximately 10 meter (E. M. Vieira & Monteiro-Filho, 2003). The canopy height of woodland savanna is low, therefore only two categories can be made in this habitat. The magnitude of the effect of trap height is very different from site to site, influenced by the present amount of fully arboreal species (E. M. Vieira & Monteiro-Filho, 2003). To get a biodiversity indication that is as complete as possible, the division of traps over different vertical layers is strongly recommended (Barnett & Dutton, 1992).

Trap height in the small mammal research of Chimbo and its effect on measured diversity

In the study of Chimbo traps are mostly placed on the ground. Only five out of hundred traps are placed above ground, at a height of approximately 1,50 meter. The importance of these above ground traps is emphasized by the captures of *Graphiurus kelleni*. This is an arboreal species, which was captured three times, all of them were in traps above ground. The low number of above ground traps can have caused an underestimation of the species richness. Besides, there is no consistency in the horizontal position of the above ground live traps, which makes statistics difficult.

The effect of the marking technique

When an animal is captured, researchers identify the species. This is done on the basis of appearance and the length of different body parts. After this, the animal will be marked and placed back at the same location where it was captured. Marking is done so that the animal can be recognized again if it is recaptured. This also prevents double counting of individuals. This chapter starts with the description of seven general marking techniques and information concerning the most important considerations when choosing a marking technique. This will also imply advantages and disadvantages of the techniques. Then, the effectiveness of the marking technique used in the small mammal research of Chimbo will be discussed. In this part it will become clear how a marking technique can have an influence on measured diversity.

General marking techniques

Marking techniques can be divided in two groups, temporary marks and long-term/permanent marks. Temporary marks are for example; dying, hair clipping and ear punching. Long-term marks are for example; ear tagging, tattooing, toe clipping and placing of a micro chip. (University of Nebraska, 2012). When a marking technique needs to be chosen, there are roughly four important consideration (Barnett & Dutton, 1992). The time that the mark remains visible, the costs, the skills required for the field workers and the ethical concerns. The marking techniques with a brief description and important information in line with these considerations are shown in table 1 for an overview.

Overall, skills, costs and ethical concerns are mostly lower in temporary marking techniques than in permanent techniques, since most temporary marks require minor change (Barnett & Dutton, 1992). The use of anesthesia is necessary in some permanent marks, toe clipping and chipping respectively (Barnett & Dutton, 1992).Therefore, these techniques require more skills. Ear tagging and chipping are the only techniques which can be used when there is a need to distinguish individuals, from the same species, from each other (University of Nebraska, 2012).

Table 1: Information of seven general marking techniques (Barnett & Dutton, 1992; Powell & Proulx, 2003; University of Nebraska, 2012).

Marking technique	**Description**	**Time visible**	**Costs**	**Skills required**	**Ethical concerns**
Dying	Spotting the animal with some dye.	3 to 4 days	Low	No specific skills needed	Can influence survivorship by enhancing predation
Hair clipping	Clipping a small piece of fur, anterior of where the tail attaches to the body.	Two to four weeks	Low	No specific skills needed	Influence on survivorship is unknown. For example whether there is enhanced predation or not.
Ear punching	Punching a hole in the ear, approximately 3 mm from the edge of the ear pinna.	5 months	Middle	The use of a punch device, which is relatively easy to learn	Can cause bleeding of the ear Can lead to pain
Ear tagging	Applying a metal or plastic tag, approximately 3 mm from the edge of the ear pinna.	Permanent. However, sticks sometimes to the vegetation or is pulled out by other animals during grooming.	Middle	The use of a tag applicator, which is relatively easy to learn	Tags can cause infections. May inhibit grooming which leads to plagues of mites and ticks.
Tattooing	Tattooing ears and/or toe pads with a microtattooer.	Permanent	High	The use of tattoo equipment. This requires lessons of a manufactor.	It can cause a lot of stress for the animal, because of the performing time
Toe clipping	Clipping of a toe, with on maximum one digit per extremity.	Permanent	Middle	Be able to work with anesthesia and blood-stopping agents.	When anesthesia is used, pain will become of lower concern. Leads to bleeding of the animal. May influence survivorship.
Microchip	Transponders placed in the shoulder area. Identification can be done with a microchip reader.	Permanent	High	The chipping technique, which requires expertise.	May wander under an animal's skin Can lead to an infection at the site of implant

<u>The marking technique used in the small mammal research of Chimbo and its effect on the measured diversity</u>

The hair clipping marking technique is used in the small mammal research of Chimbo. Costs and skills were taken as the most important considerations. Chimbo wants to accomplish this survey with the help of volunteers, students and locals (Silvavirconsultants, january 2014). Therefore, the needed skills have to stay low. Also, as in many studies, costs of the total research have to stay as low as possible.

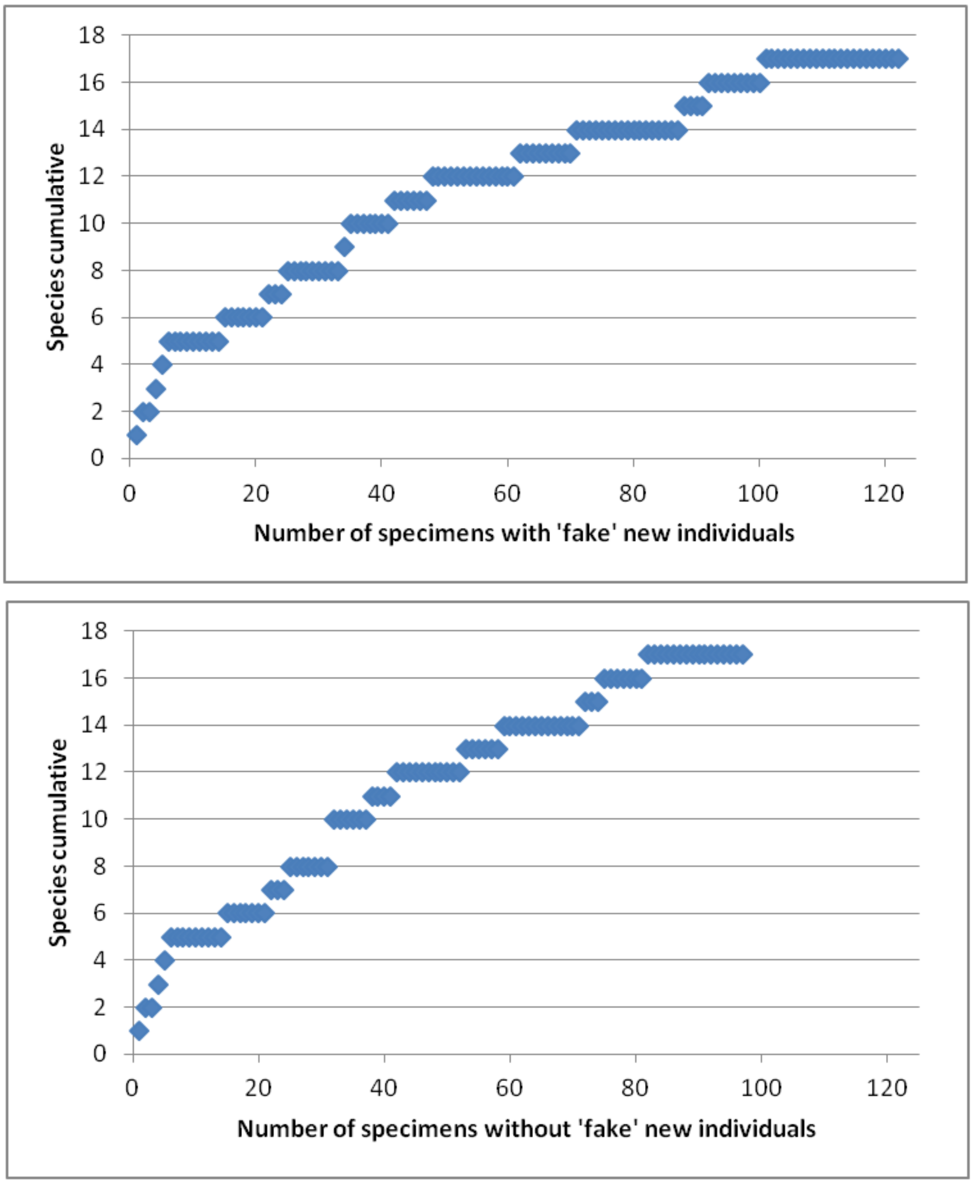
The small mammal research has a research time of one year. However, as mentioned in table 1, a hair clipping mark will remain visible one month at the most. To make the mark visible for a longer time, hair has to be clipped again (Silvavirconsultants, january 2014). To do this, the animal has to be recaptured within this month. In case this does not happen, the animal will be seen as a new individual next time it is captured. For clarity, individuals like these will be called 'fake' new individuals from now on.

At first, this seems to have no influence on the aims of this study because, species richness is used to express diversity. The number of species (species richness) will not be affected by 'fake' new individuals. However, a species accumulation curve, made to see if the samples reflect the target species present in the area, will be influenced. To be able to understand this influence, some characteristics of a species accumulation curve should be known.

In a species accumulation curve, species cumulative is plotted against specimens captured (Bâ et al., 2013; Magurran, 2004). Recaptures are excluded in this graph, since specimens are captures of different individuals. So, a specimen always cause a step to the right on the x-axis. The y-axis stays on the same height when the specimen is a species which was already captured during the research or will go one step upwards when the specimen is a new species. A community is well sampled when this curve reaches a flattened shape (a plateau) (Magurran, 2004). Meaning that every specimen captured from then on will, most likely, not be a new species.

A 'fake' new individual and therewith a specimen will also cause a step to the right on the x-axis. However, this 'fake' new individual will never lead to an upwards step on the y-axis. Since this individual is actually already captured once and will therefore never be a new species. So, all the 'fake' new individuals will flatten the species accumulation curve. As said, it is precisely this shape which indicates the completeness of the samples. In other words, the community seems better sampled than it actually is when marks are lost during the research time.

Figure 4 shows two graphs to visualize this concept. The first graph (A), is a graph made with the original data of the small mammal research of Chimbo. In these data it is likely that there are lost marks, so there are 'fake' new individuals which can influence the curve. The second graph (B) is based on the following thought experiment: Imagine that after one month, which is in correspondence with the moment that 25 specimens are captured, one out of four captured specimens is actually a 'fake' new individual (After one month tags start to disappear, so some recaptured individuals will be identified as new individuals). Removing of these 'fake' new individuals, results in graph B. Keep in mind that the ratio one out of four is just chosen to illustrate the concept, since the real number of 'fake' new individuals is unknown. When both curves are compared, it will become clear that 'fake' new individuals cause flattening of the curve.



A

B

Figure 4: A illustration of the influence of 'fake' new individuals on a species accumulation curve

The disappearance of marks has another important influence, namely on measured species abundance. Since 'fake' new individuals are registered as new individuals, the amount of individuals per species will be overestimated. This increases the obtained values of diversity indices. Which in its turn plays a broader role in the set up of protection programs. This point is mentioned because it should not be overlooked in studies that include abundance in their measurement of species diversity. However, this is no issue in the small mammal research of Chimbo, because therein diversity is only expressed in species richness.

The effect of species' specific characteristics; lifecycle, home range and dispersal patterns

The trappability of small mammals depends on variety of factors like trap type, bait type, season, weight of the animal, habitat etcetera (Krebs & Boonstra, 1984; M. Vieira, Grelle, & Gentile, 2004). The influence of these factors can all be related to species' specific characteristics. Three important characteristics having influence are the lifecycle, the home range and dispersal patterns (Coetzee, 1975; Collins & Wallace, 1990; Letnic, 2002; Schradin et al., 2010). However, for most species these characteristics are unknown. This makes it hard to take it into account in a diversity study. In this chapter the influence of these three characteristics will be discussed as complete as possible with the existing literature on this subject.

Lifecycle

The lifecycle of *Mamstomys natalensis* is very well known. Since, this species is regularly investigated in several regions of Africa related to its high risk in spreading Lassa virus, which is a serious threat for humans (Coetzee, 1975; Lecompte et al., 2006). Effects of the lifecycle of small mammals on measured diversity will therefore be discussed, with the lifecycle of *Mastomys natalensis* as starting position.

The average age of death of *Mastomys natalensis*, due to natural causes including both diseases and old age, was determined in two laboratory stocks at the Medical ecology Centre in Johannesburg. An average age of death of respectively 395 and 487 days was found (Coetzee, 1975). So this rodent has a lifecycle of a little bit more than one year. The age at first litter can be regarded as 94 days (Coetzee, 1975). The gestation period is 23 days, with a 25-day interval between litters. The breeding season has a duration around 10 months. Making a small calculation with these numbers, gives the insight that four generations can be born in one year (Coetzee, 1975). In other words, the animal has a short lifecycle and fast replacement by offspring.

The short lifecycle results in the capture of different generations in long term studies. While new generations are captured over time, older generations disappear. Species abundance is mostly determined at the end of a study by taking the total number of individuals captured per species during the research time (Magurran, 2004). Therefore, this total includes all generations. However, these generations did not really exist simultaneously. This causes an overestimation of species abundance.

The lifecycle includes different stages of life. These different stages are in correspondence with different trappabilities. Juveniles are for example less likely to be captured than adults (Umetsu, Naxara, & Pardini, 2006). Also the reproductive status strongly influence trappability (Jensen, 1975). The effect of the reproductive status can cause an increase in trappability of specific species during specific times of the year and on the contrary decreases at other moments (Coetzee, 1975). This can ultimately ensure that species are over looked in studies covering only a part of the year. In other words, this can lead to a lower measured species diversity.

Visibility of offspring in data of Chimbo and its effect on measured diversity

The collected data of Chimbo in the dry season, covers five months of the year. In these five months, only 10 out of 121 captured individuals are juveniles. This low trappability of juveniles is in correspondence with the literature described above. Reduced trappability due to the reproductive status of the target species cannot be determined by the data. However, the possibility of overlooking species is prevented by surveying during a full calendar year. Therefore, species diversity is measured as optimal as possible. The effect of capturing more generations does not matter in this study, because species abundance is not a direct variable of interest.

Home range

The home range of an animal is determined by its space used during day to day activities, which occupies only a part of the total available environment (Giuggioli, Abramson, Kenkre, Parmenter, & Yates, 2006). Both the shape and the size of a home range are important in placing transects in diversity studies. A transect needs to be efficient in capturing the most possible different individuals and therewith species. This efficiency decreases for example when more than one transect covers the home range of one individual. Therefore, the optimal distance between transects depends on the home range size and shape of the target species. It is also possible that the orientation of the transect influence the optimality. When home ranges at a certain place have for example an elongated shape (Metzgar, 1973), caused by any factor whatsoever, the transect will lead to more individuals when it is placed perpendicular on these home ranges than when it is placed in parallel (figure 5). Therefore, information on how home range size and shape are established will be given below. Subsequently, it will be discussed whether this knowledge can be used to choose an optimal position for transects in advance.

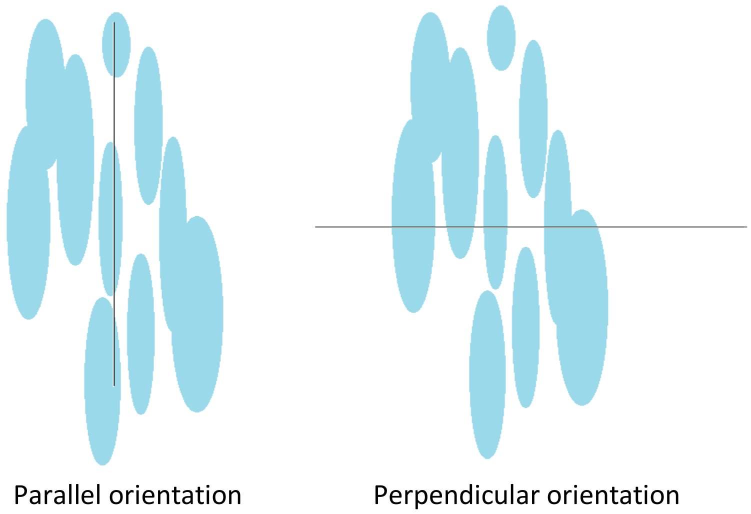


Figure 5: Sampling efficiency of a transect, caused by its orientation with respect to home range shape. Home ranges are visualized with blue spots and transects with a black line.

The home range size, shape and location depends on the state of the individual and the conditions of the external environment (Börger, Dalziel, & Fryxell, 2008). The best known influences are resource availability, reproductive status, population density, mating system and climate season (Borremans et al., 2014; Rémy et al., 2013; Schradin et al., 2010). These variables themselves are also correlated to each other. For example, mice in a resource rich environment will have higher population densities and have a polygynous mating system (Guichón, Borgnia, Righi, Cassini, & Cassini, 2003). Schradin *et al.*, (2010), have tried to put direct and indirect influences on home range size, specifically, in a scheme (figure 6).

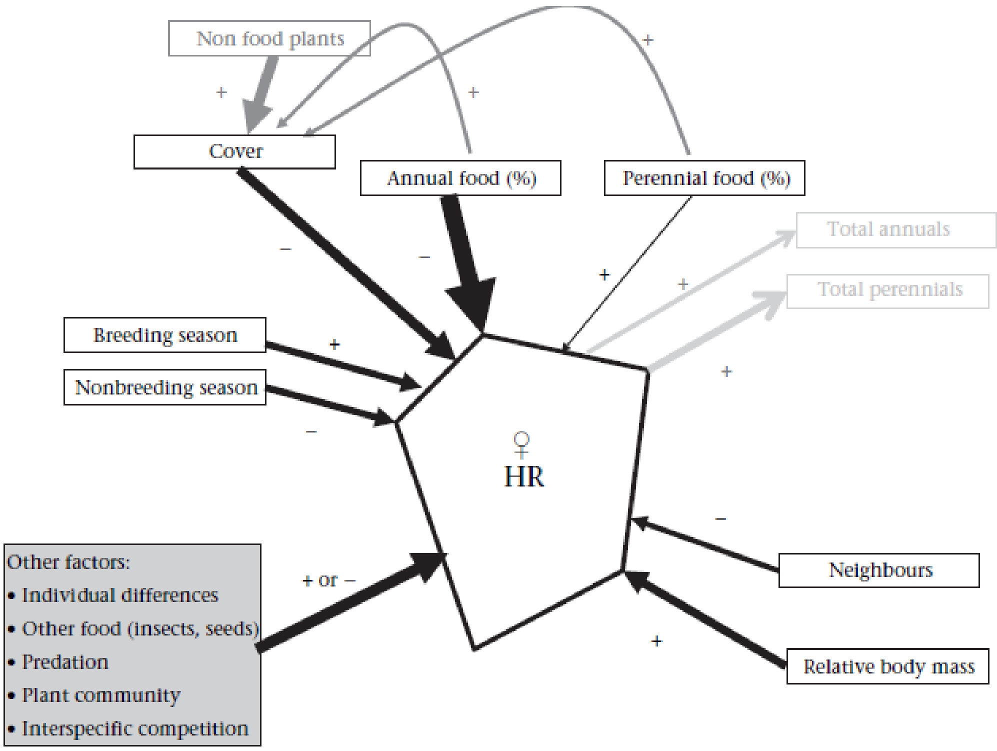


Figure 6: The overall direct and indirect influences of the most important variables on home range size. A minus corresponds with a decrease in home range, where a plus corresponds with an increase. This scheme is made with data of a striped mice, *Rhabdomys pumillo,* in south Africa by Schradin et al., (2010).

Some of these general influences are further explained below, to give a better understanding how the positive and negative effects, in figure 6, are established.

- The effect of resource availability on home range size and overlap depends strongly on the sex of the animal. Energetic demands of the female reproduction system are high, therefore reproductive success is strongly limited by food. So, a female's home range should show a strong correlation with resource availability. Males are on the contrary are dependent on the females. Therefore, their space pattern is strongly influenced by the female pattern. In Rémy *et al*., (2013), the effect of three types of food dispersal (dispersed, clumped and variable) on home range size and overlap is investigated for the species *Myodes glareolus* (figure 7). Females in the clumped treatment had significantly more overlapping home ranges than in the other two treatments. Home range size was not affected by the treatment in neither sex. This last point is in contradiction with the expectations made before by Rémy *et al*, (2013). Because data is possibly affected by population density effects and the presence of fences, this result should be approached with caution. (Rémy et al., 2013)

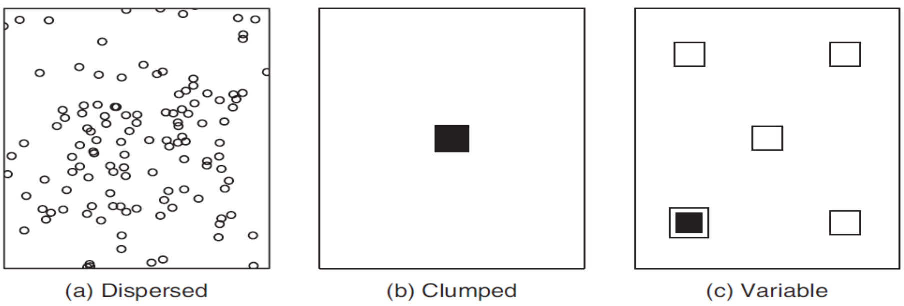


Figure 7: Three types of food distribution, dispersed, clumped and variable. In the variable treatment a clump of food is placed on one of the five preset sites twice a week. (Rémy et al., 2013)

- Differences in quality of the available resources also influence the home range. Annual plants have more proteins than perennial plants, so annual plants are a better resource. Therefore species living at places with more annual plants have smaller home ranges compared to species living at places with more perennial plants. (Schradin et al., 2010)
- The age of an individual is positively correlated with home range size. In the study of Borremans *et al.*, (2014), for example, a subadult *Mastomys natalensis* had a home range of approximately 546 m^2, where an adult had a range of 636 m^2 (Borremans et al., 2014).
- The breeding season leads to an increase in home range size. Possibly caused by higher energy demands (Schradin et al., 2010). In discrepancy with this Borremans et al., (2014), showed a decrease in home range size of both sexes during breeding season. This effect was probably influenced by the increasing population density, during this period. Higher population density cause significantly smaller home ranges (Borremans et al., 2014).

All the information given until now, reflects directly the problem when dealing with home ranges. Namely, each factor influences the size, shape and location of the home range in a different way and the influence of the factors itself are also variable depending on the circumstances. This makes it hard or even impossible to describe a general home range (Börger et al., 2008). For example, the average home range size of *Mastomys natalensis,* found by Borrremans *et al.,* (2014), can only be used as a very rough estimation for other research studies. Since these areas have different resources, climate conditions, plant species etcetera. Therefore, transect distance and orientation can hardly be based on the home range of the target species.

The visibility of the home range effect in data of Chimbo and its effect on measured diversity

The transects in the small mammal research of Chimbo are positioned at a distance of 500 meter from each other. According to the data, some individuals are captured in traps of two different transects. Chimbo uses a marking technique, which cannot be used to distinguish individuals from the same species from each other. Therefore, only striking individuals, caused for example by having a half tail, can be distinguished from the others. This makes it hard to determine to what extent this happened and therefore it is also not possible to say to what extent this has affected the measured diversity. However, when it did happen, it could be caused by two reasons. The size of the home range is large enough to cover two transects or the home range is moved over time. The latter belongs to dispersal patterns, which will be discussed further below.

Dispersal patterns

One-way movement of an individual from a home range to a new, non-overlapping, home range is defined as dispersal (Pocock, Hauffe, & Searle, 2005). To determine dispersal patterns of target species, they need to be traced. However, tracing small mammals is hard, because it is not possible to trace such mostly nocturnal and small animals by watching (Lemen & Freeman, 1985). A technique like fluorescent spraying combined with capture-recapture data can partly overcome this problem (Lagos, Contreras, Meserve, Gutiérrez, & Jaksic, 1995; Lemen & Freeman, 1985). Spraying captured animals with a fluorescent pigment will lead to a fluorescence trail made in the night of the animals release, because the pigment is brushed against the vegetation (Lemen & Freeman, 1985). This trail can be followed with an ultraviolet lamp, the day after. The capture and recapture data from grid-designs can also give dispersal information (Lagos et al., 1995). However, it stays hard to trace longer distances. Most existing literature therefore focuses on dispersal patterns at specific places affected by big disturbances like fires. Because, knowledge of the dispersal effect of these disturbances is of great importance for the set up of protection programs. The impact of a fire in a savannah area can be very different from site to site (Collins & Wallace, 1990). Important factors in grassland fires are changes in litter and standing dead vegetation layers after the fire and the climate conditions and the present species before the fire. Some species are negatively correlated with fire, meaning a decrease in population density, caused by for example migration to other places (Collins & Wallace, 1990). Other species on the other hand are positively correlated with fire, meaning an increase in population density (Collins & Wallace, 1990). *S. youngsoni* for example preferred regenerating habitats after fires in Australia (Letnic, 2002).

More general patterns of species driven by for example climate season or breeding season are on the contrary much less known. There are speculations that small mammals in savanna areas retire in patches of gallery forest during the dry season (Silvavirconsultants, january 2014). This is based on the assumption that these animals have an insufficient vegetation cover in the grassland during this period. However, there are no studies to confirm this.

Dispersal patterns described above can directly affect trappability in diversity studies. Disturbances can cause either the capture of more species or the capture of less species. This depends on the species' specific reaction on a certain disturbance. If speculations about retirement of small mammals to gallery forests, in savanna areas during dry season, are correct, species can be overlooked during diversity studies in these regions. This can be solved by also placing traps in these gallery forests or by capturing during different parts of the year.

Possible dispersal during the small mammal research of Chimbo and its effect on measured diversity

There was a fire at one research location in the small mammal research of Chimbo, approximately three months after the start of the study. This can have influenced the number of species and/or species composition on this specific location. Since there is no general influence (described above), it cannot be said to which extend this matters in the total measurement of the diversity. Besides, huge areas, just behind the research locations were clearcutted during the dry season. It is not known, to my knowledge, which influence this has on the migration of small mammals. However, it is imaginable that this can result, like fires, in migration of small mammals to other places.

The research locations of Chimbo are situated at different habitat types. Which reduces the chance to overlook species, caused by possible habitat preferences in relation with seasonality. This reduction is enforced by capturing small mammals during a whole calendar year. In other words, dispersal patterns have, most likely, influenced the measured diversity, however it is hard to determine the impact.

Trap disturbances

The first three chapters all specified on influences related directly or indirectly with small mammals, the animals of interest. However, in the field there are more animals. Although these are not target species, they can still be attracted to the live traps (Barnett & Dutton, 1992). This can cause additional captures and/or disturbances. When the number of additional captures stay low, since the traps are specifically created for the capture of small mammals, this is no problem. Disturbances on the contrary can strongly influence the obtained data of the target species (Hooven, Black, & Lowrie, 1979). Therefore, two common disturbances described in small mammal live trapping studies will be discussed in this chapter. Beginning with disturbances by larger animals followed by disturbances by ants. From both, causes and solutions from literature will be discussed followed by specific disturbances in the research of Chimbo and the impact of their solutions on the measured diversity.

Disturbances by larger animals

Larger animals, mostly carnivores, are not only attracted by the bait, but also by captured mice. Disturbances are caused by injuring or killing of captured mice. This results in a lower number of identifiable captures, which can influence measured diversity. Skunks were for example decreasing the amount of captures in the research of Hooven *et al.*, (1979), where raccoons did the same in the research of Layne, (1987). There are several ways, described in literature, to avoid this: Putting out a plate of food for the carnivore (Barnett & Dutton, 1992), repeatedly moving of the traps (Barnett & Dutton, 1992; Watson & Watson, 1985), removing the problem animals (Watson & Watson, 1985) and a device which protects the live traps from predators (Hooven et al., 1979). Every solution has some disadvantages. A plate of food can attract scavengers and therewith deter the small mammals. Moving the traps can be dramatic for the study's design. Removing/capturing can strongly influence the predator-prey interaction. In addition there are the risks of capturing these animals and then there is the question where to put the animal when captured? (Atkinson, 1997) Making a device like a small fence with the mesh size big enough for the target species, but too small for the non-target species, seems to be the best solution, because this interferes as little as possible with the results. The most important disadvantage is cost and effort.

An example of the effectiveness of this solution can be given by the study of Layne, (1987). During eight years, from 1969 to 1977, there was a progressive increase in trap disturbances, reaching 45.9% in 1977. Raccoons were assumed to cause the major part of these disturbances. Therefore, an enclosure which exactly fits a Sherman live trap was created (figure 8). By making it as small as possible, costs stayed as low as possible. These enclosures worked for nine years (1977-1986), without being damaged, thanks to the metal. The average disturbance percentage measured in these years was only 1%. It can be concluded that this investment will pay off during long run studies (approximately 10 years). (Layne, 1987) These enclosures need to be tested in other areas to measure the effectiveness of protection against other carnivores. However, costs and effort remain a problem with this solution, especially with short term studies.

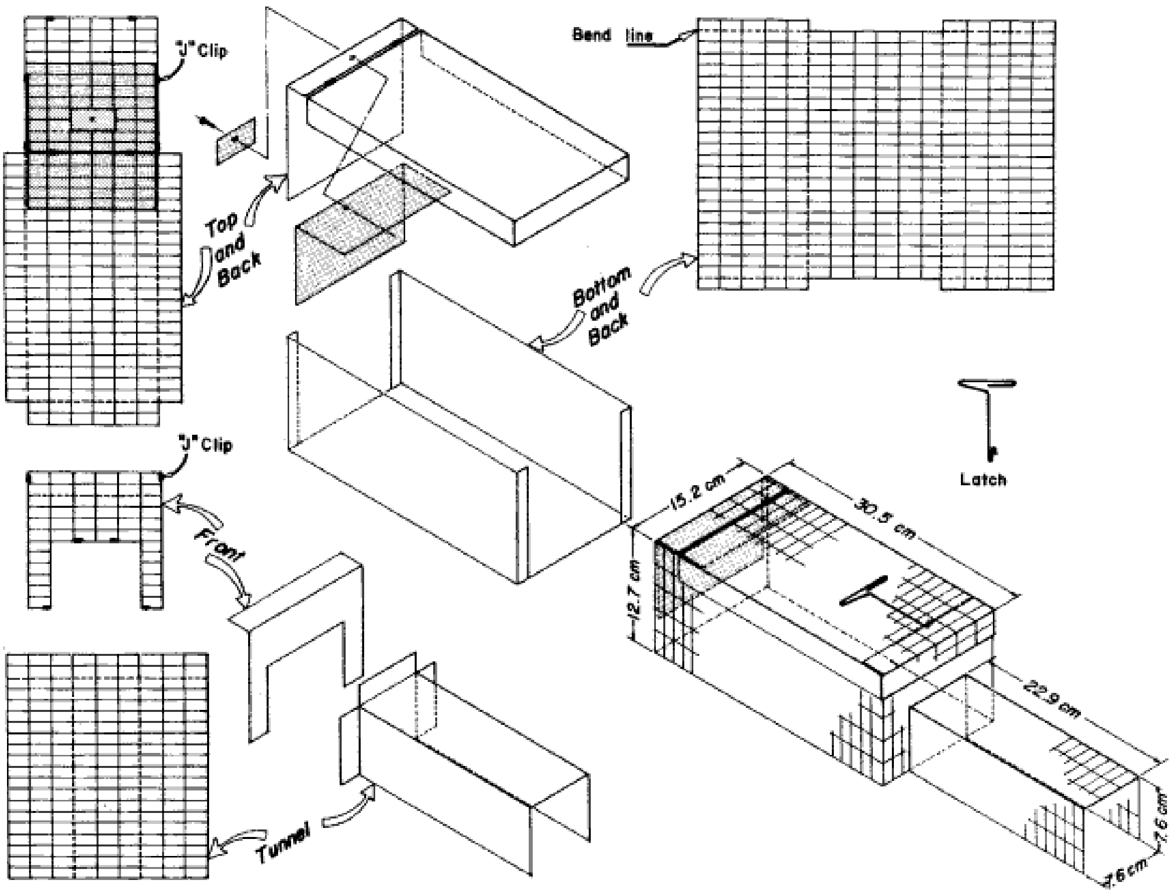


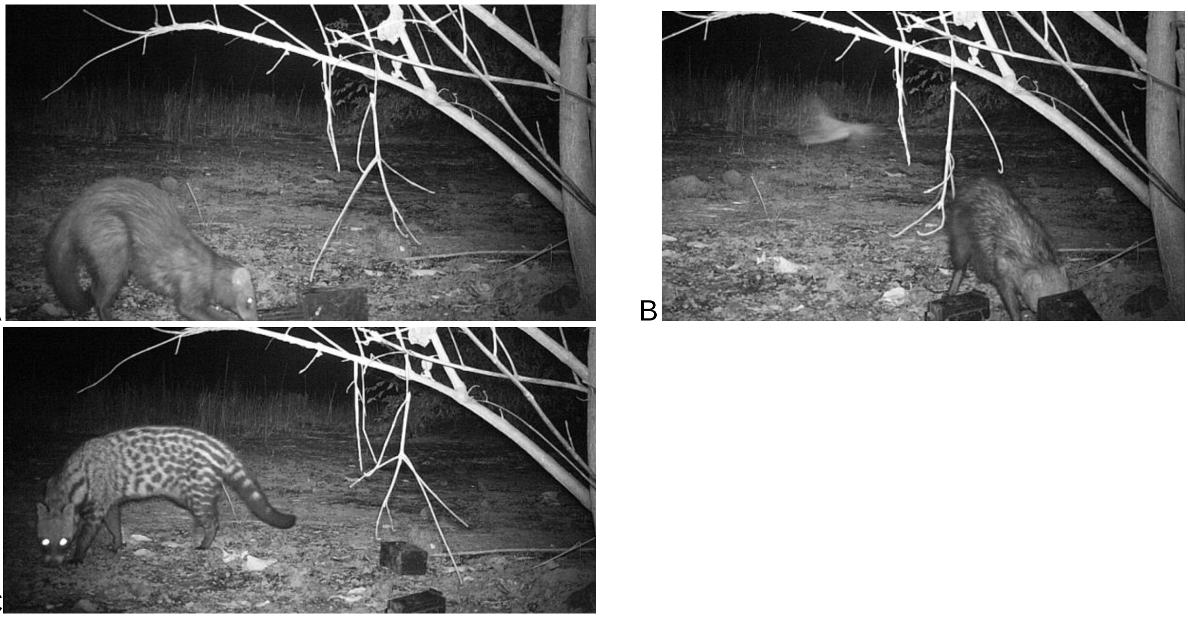
Figure 8: Enclosure to protect animals captured in Sherman traps from predators. (Layne, 1987)

<u>Disturbances by larger animals in the study of Chimbo and its effect on measured diversity</u>

In the study of Chimbo disturbances by larger animals occurred in a constant manner at one of the five research locations and occasionally at other research locations. Traps were found upside-down, closed and/or dismantled during morning controls. Cameras filmed mongooses which learned themselves to gain the bait inside the trap, by pushing, pulling and rolling of it (figure 9). Besides mongooses, a civet cat was photographed during a night. The trap where this camera was positioned was already deconstructed by a mongoose earlier that night, so there are only pictures of a civet cat passing.

To solve this problem, the location which was constantly disturbed was removed, the materials were used to make another location as replacement. The position of this new location has another habitat compared to the old one. This was done with the purpose to immediately rise the capture of new species for the research. The new location indeed lead to the capture of new species, which increased measured species diversity. However, it is important to keep in mind that the habitat of the original location is possibly not fully sampled. Besides, nothing was done against the occasional disturbances at the other locations, which has possibly lead to a lower number of captures. Which in its turn can have lead to a lower number of species captured.

In addition to carnivores, also herbivores, like cows and sheep caused serious problems by running over the traps. In Guinea Bissau, shepherds walk with their cattle through gallery forests at the end of the dry season. Because the last remaining nutritious food is left in these forests. These are also the research locations for the small mammal research. It is hard to avoid this. A small fence around the live trap can be a solution, however this is expensive. Therefore, nothing was done to solve this problem. This has lead to a lower number of captures and probably therewith measured species richness.



A B

C

Figure 9: Pictures of camera traps placed at location one. These pictures are made during the night of 22-2-2014. Pictures A and B show a mongoose that deconstructs a Heslinga life trap, picture C show a civet cat passing.

Disturbances by ants

Ants can remove the bait and/or mutilate captured mice. Both eventually lead to the identification of a lower number of individuals, which can result in a lower number of species. This problem was for example caused by fire ants in studies in the Southern United States (Kraig, Roels, & Thies, 2010). Therefore, the effectiveness of chemical repellents in deterring fire ants from Sherman live traps is investigated (Kraig et al., 2010). Some repellents were very effective, did not interfere with the number of captures and were not washed away after a heavy rain (Kraig et al., 2010). Repellents avoid both, removing of bait and mutilated mice. This sounds very promising, however these repellents are specific for fire ants. Therefore, it should be interesting to investigate a mixture of repellents, which are effective for more types of ants. In that way, this can solve the problem for a lot of other studies with live traps.

When ants cause only problems with the bait, there is another possible solution, namely the use of a special type of bait. This solution comes forward from a study of Atkinson, (1997). Bait composed of 1 cm^3 balls of porridge, made from maize meal and boiling water in a volume ratio of 1:3, mixed with peanut butter to a consistency of pastry dough was used a prior in the study of Atkinson, (1997). From 100 baited live traps, bait was removed by ants in 47 traps within 24 hours. Therefore, another type of bait was investigated. This new bait was made as follows; 250 gram peanut butter was diluted in 1 liter warm water (=60°C). Cotton wool, with the size of approximately 2 cm cubes, was soaked in this mixture. This soaked cotton wool was then moulded in 1 cm^3 balls and dried in an oven at 40 °C for circa nine hours. This procedure eventually lead to balls with a soft toffee texture and appearance. These balls were clipped on the trigger mechanism of the traps. Because the cotton is uneatable for ants, the bait was not removed anymore. However, peanut butter on the surface of the balls, was still being eaten. This problem was solved by coating the balls at the end with a 1 millimeter suspension of the peanut butter mixture made in the first step of the preparing process. This coating was repeated after every night of trapping. This type of bait was very effective and did not interfere with the number of captures. The advantage of this solution is that it is effective against bait removal of every type of ants. However, it is a very time consuming process and is not effective against mutilation of captured mice. (Atkinson, 1997)

Disturbances by ants in the study of Chimbo and its effect on the measured diversity

In the small mammal research of Chimbo, army ants were disturbing live traps. These ants patrol through an area, till they find a prey (Gotwald Jr, 1995). Then a colony which can be up to a million ants, attack the prey (Gotwald Jr, 1995). In this case the prey was a mouse captured in a live trap. This problem was solved by immediately closing the specific research location during one week. This solution is based on the idea that these ants will leave to another area, when there are no preys left at the research location. This solution worked well. There were no more disturbances by army ants thereafter. Since this solution was performed immediately after detecting two live traps crowded by army ants (figure 10), only these two captures were lost during the whole study through army ants. Therefore, it is most likely that this did not have influenced the measured species richness.

Figure 10: Photographs of two live traps crowded by army ants.

Discussion

The effect of sampling on measuring small mammal diversity is investigated in the light of four aspects of capturing small mammals; Sampling location, the marking technique, species' specific characteristics and trap disturbances. The most important conclusions will be given below, followed by ideas for future studies. Conclusions and recommendations in relation with Chimbo's research will be given underneath.

Sampling location was divided in trap spacing and trap height. Overall trap spacing is done in a grid or in a transect. A transect leads to the capture of more species than a grid, with the same trap number. Therefore, a transect is better for a diversity study. However, when the aim of a study is related to movement patterns a grid can give more information. Placing of the traps at different heights is essential to get a complete biodiversity indication, since there is a clear vertical stratification in the occurrence of small mammals in forests.

Different marking techniques have different advantages and disadvantages in relation with four common considerations; time visible, costs, skills required and ethical concerns. The time of visibility of the mark needs to be at least the same as the research time. Otherwise, the community seems to be better sampled than it really is according the species accumulation curve and species abundance cannot be measured.

Species' specific characteristics was divided in the lifecycle, the home range and dispersal patterns of the investigated animals. The short lifecycle of the target species results in the capture of more generations during long term studies, which can cause an overestimation of species abundance. Therewith, different life stages have different trappabilities. Therefore, species can be overlooked during certain times of the year. The home range size, shape and location can influence the capture effectiveness of transects, however the impact is unknown. No general home range size, shape and location for small mammals can be given. Since this is determined by too many direct and indirect influences of both, the external environment and the state of the individual. Transect orientation and distance can therefore not be adjusted to it. Dispersal patterns are mostly investigated in relation with big disturbances. Disturbances can change the number of species and/or species composition at a certain location. Which eventually influence measured diversity, either positively or negatively.

Trap disturbances by other animals was divided in larger animals and ants. Larger animals can decrease the number of captures by dismantling live traps and/or by mutilating or killing captured small mammals. This can lead to a lower measured diversity. Placing of an enclosure around the live traps is the best way to solve this problem, however this is expensive and costs a lot of effort. Ants can also decrease the number of captures. This is caused by the removing of bait and/or mutilating of captured small mammals which makes them unidentifiable. The use of repellents can overcome both, but for the moment there are only repellents specific for fire ants. Removing of bait can be thwarted by the use of cotton soaked with peanut butter diluted in warm water as bait. However, this is a very time consuming process.

It can be concluded that sampling has an effect on the measured diversity, which can be both positive and negative. Since sampling influence measured species abundance and species richness on the basis of four general aspects; sampling location, marking technique, species' specific characteristics and trap disturbances.

The influence of the sampling location was very clear in literature (Barnett & Dutton, 1992; Pearson & Ruggiero, 2003; E. M. Vieira & Monteiro-Filho, 2003). It is concluded that a transect design is the best, when the aim of the research is to measure diversity. However, when choosing a transect, information about movement patterns and home ranges is lost (Pearson & Ruggiero, 2003). Since most of these studies are a part of a broader purpose, the set up of ecological protection programs (Datiko & Bekele, 2013; Decher et al., 2001; Makundi et al., 2010), it can be useful to have information of all species richness, species abundance, movement patterns and home ranges. Therefore, it should be interesting to investigate if another design, made on basis of the current knowledge of grids and transects, can give this. I would propose, for example, an open triangle design. An open triangle, should theoretically have the benefit of a large effective sampling area of a transect and the benefit of visualization of movement of a grid. This can be seen as follows: When using 25 traps a transect will have 25+56 effective units (Pearson & Ruggiero, 2003), a grid has 25+24 (Pearson & Ruggiero, 2003) and an open triangle has 24+54 (figure 11). So in this way, the benefit of a large effective sampling area is maintained when using a triangle configuration. Animals still cross at least two lines of traps, when going into a certain direction, which leads to information about movement. An open triangle is probably better than an open rectangle of 25 traps, because an open triangle has less unsampled area inside (figure 11). Therefore the chance that an animal, living ‘in the triangle’ is more likely to be captured once at the boundaries, formed by the transects, than an animal living ‘in a square’. Besides, the effective sampling area in a triangle is larger than in a rectangle. As far as my knowledge stretches, there is no literature about this type of designs.

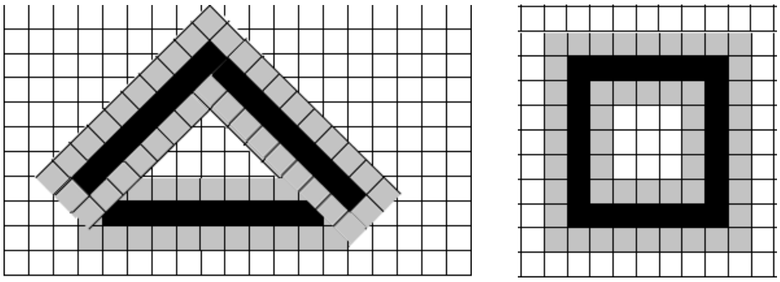


Figure 11: Schematic figure which shows effective trapping area sampled by an open triangle arrangement and by an open rectangle. Way of visualization based on Pearson & Ruggiero, (2003).

It became clear that species' specific characteristics like home range and dispersal patterns are not only influenced by the species, but also by the external environment (Börger et al., 2008). This subject is hard to handle, because there is no information of all factors (species and external environment) at a certain location. However, it is interesting that home range size, for example, is so strongly depended on its external environment (Borremans et al., 2014; Schradin et al., 2010). Maybe, it is especially this what can make small mammals the ideal bio-indicators in the future. Therefore, more research on specific species and their relation with ecosystem properties as vegetation density, plant species, ratio annual/perennial food etcetera can be useful (Carey & Harrington, 2001). This also includes further research on general dispersal patterns, beginning with the investigation of speculations like the retraction of small mammals to gallery forests during the dry season. Though, research on dispersal patterns remain hard, when tracing of small mammals on longer distances stay difficult.

Trap disturbances caused by larger animals and ants is mentioned in a lot of small mammal studies (Atkinson, 1997; Barnett & Dutton, 1992; Hooven et al., 1979; Kraig et al., 2010; Watson & Watson, 1985), however only a few took precautions or integrated solutions. This is probably caused by the costs related to the effectiveness of the existing options. For example, the use of an enclosure around traps, against larger animals, is expensive (Layne, 1987). Therewith, it is only tested with raccoons as disruptors and it can therefore not be guaranteed that it is effective against other larger animals (Layne, 1987). Another example, is the use of repellents against ants, this is also expensive. Therewith, it is only effective against fire ants and therefore needs to become less specific to be useful in every small mammal study (Gotwald Jr, 1995). In other words to make precautions against trap disturbances more accessible, research on improving and/or testing of existing ideas is necessary.

Recommendations for Chimbo

Below a summation of elements of the small mammal research of Chimbo with advantages and disadvantages in relation to the measured species richness will be given. This will be followed by an explanation of the most important recommendation for this study.

- Placing live traps in a transect is the best way to capture as much as possible different species. Since a transect has more effective sampling area and crosses more unique microhabitats than a grid. Therefore, this is the best placing design for live traps for the research.
- A trap distance of 5 meter is very reliable in a transect. However, a distance of 10 meter is also reliable and can therefore be considered. The advantage of this is that the transect becomes twice as long with the same amount of traps. Therefore the transect will cross a larger area, which improves the chance of capturing different species.
- It is good that traps were placed both on the ground and above ground. However, only one trap out of twenty was placed above ground at every location. It is recommended to place more traps above ground. This will result in a bigger chance to capture arboreal species, besides a more even distribution of traps facilitates statistics on capture differences. Shifting of all live traps, in current transects, to a position approximately 1,5 meter above ground for one month, is worth considering. In this way there is no new material required and it is optimal to make static comparisons between captures on the ground and above the ground, since all other variables are kept as equal as possible.
- Hair cutting is a very temporary marking technique. Therefore it is not recommended in long term studies. Marks will be lost and recaptures will be seen as new individuals. This makes the collected data only useful in the determination of the number of species. However, knowledge of species abundance can be very worthy in the setup of protection programs for the area. Therefore, ear tagging is a better marking technique. This technique complies with the most important requirements for the research; permanent mark, costs stay relatively low, every individual has an unique number and relatively easy to apply.
- The research time of one calendar year is a good choice. This leads to captures from every climate season, therewith the chance to overlook species will be reduced.
- It is useful to consider protection of live traps against disturbances of larger animals in the form of enclosures. Especially since this is a long term study, which makes the chance of disturbances bigger. This is enforced by the fact that big disturbances are already faced in the first half year of the research. Therefore the benefits of enclosures can probably outweighs the costs.

Overall it can be concluded that Chimbo measured the species richness very optimal. A change which can result in a huge improvement is the use of another marking technique, like ear tags. This marking technique prevents the capture of 'fake' new individuals, which biases the species accumulation curve. Therewith, species abundance can be measured. The measurement of species abundance is at the same time a second improvement. With information about both, species abundance and species richness, measured species diversity gets more value. Which in its turn can lead to a more optimal set up of protection programs, the broader aim of Chimbo Foundation.

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