ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
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FLOCK SIZE, HABITAT ANALYSIS, DIET COMPOSITION AND GENETIC DIVERSITY OF ANKOBER SERIN (*SERINUS ANKOBERENSIS*) IN SEMIEN MOUNTAINS NATIONAL PARK AND GUASSA COMMUNITY CONSERVATION AREA, ETHIOPIA

BY

ABEBAYEHU DESALEGN

A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES OF THE ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGY (ECOLOGICAL AND SYSTEMATIC ZOOLOGY STREAM)

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June, 2017

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Flock size, habitat analysis, diet composition and genetic diversity of Ankober Serin (*serinus ankoberensis*) in Semien Mountains National Park and Guassa Community Conservation Area, Ethiopia

Abebayehu Desalegn

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Abstract: Ankober Serin (*Serinus ankoberensis*) is an endemic bird limited in its distribution to certain high altitude ranges of Ethiopia. The present study was aimed to study the flock size, habitat disturbance, habitat analysis, diet composition and genetic diversity of the species in Simien Mountains National Park (SMNP) and Guassa Community Conservation Area (GCCA). Five flocking sites (ranging from 0.25–0.5 km^2) were identified in each study area. The level of habitat disturbance was categorized as low, medium and high. Plant species diversity, composition, vegetation cover and height of Ankober Serin's habitat were assessed for habitat analysis in two accessible flocking sites per study area. Diet analysis using fecal droppings was used to determine the diet composition of Ankober Serin in the two accessible flocking sites. The intra and inter-population genetic diversity was determined from five microsatellite markers. Flock size difference between wet and dry seasons for the combined data from both study areas was also significant (t (38) = 3.053, α = .004). Similarly, there was significant difference of mean flock size among all the flocking sites ($F_{9, 39}$ = 6.882 p=0.000). Level of disturbance was negatively correlated with flock size (r=-.876 at 0.01 level) in both study areas. The flocking site with *Festuca* spp. and *Helichrysum* spp. dominated habitat in SMNP had the highest flock size (96.7±34.7). There was no direct relationship between vegetation diversity and flock size. Also, there was no significant correlation between flock size and proportion of herbs (r= -.019, p= .885), bare ground (r= .030, p=.821) and shrubs (r= .006, p=.963). Vegetation heights of <30 cm constituted highest proportions in all of the foraging grounds of the flocking sites. There was positive correlation between flock size and vegetation heights of 31–60 cm (r= .624, p= 0.01) and > 60 cm (r= .577, p= 0.01) within the same foraging grounds. During the dry seasons, seeds significantly predominated the diet of Ankober Serin ($F_{1, 6}$= 324 p=0.000) and ($F_{1, 6}$= 2437.098 p=0.000) at GCCA and SMNP, respectively, while leaves were dominant during the wet season ($F_{1, 14}$= 219.842 p=0.000) and ($F_{1, 6}$= 580. 246 p=0.000) at GCCA and SMNP respectively. Three of the four molecular markers i.e., Cuµ28, Ase43 and Ase42 used in the genetic analysis resulted in significant differences between the populations of SMNP and GCCA (p < 0.05). The presence of shared alleles indicated gene flow between the populations. In GCCA, markers Cu28 and Ase42 showed significant deviations from Hardy-Weinberg equilibrium and in SMNP, the markers Ase42 and Ase43 showed significant deviations (p<0.05). The variations in flock size may be attributed to the differences in the degree of disturbance and habitat size. The predominance of leaves during the wet season could be due to scarcity of seeds during the season. The significant genetic differentiation among the two populations might have resulted from the geographical separation of the two populations by barriers such as mountains, valleys and extensive plains as well as the separation distance which is more than 360 km. The stepping-stone pattern of small migration distances might have favored the observed gene flow. The deviations from Hardy-Weinberg equilibrium in both populations might have resulted from limited population size and restricted gene flow between the populations of Ankober Serin.

Key words: Ankober Serin, Diet composition, Flock size, Genetic diversity, Habitat disturbance

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"Above all, I praise my God, my salvation."

Abebayehu Desalegn

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1. Introduction

1.1. Background and Justification

1.1.1. Taxonomy and phylogeny

The names "Passerine" and "Passeriformes" are derived from the Latin term "passer" for passer sparrows and similar small birds. Passeriformes is the largest order of birds. The arrangement of their toes, three pointing forward and one back which facilitates perching is different from other orders. Thus, some times they are known as perching birds. They are one of the most diverse terrestrial vertebrate orders. Order Passeriformes contains more than 110 families and three sub-orders (namely: Acanthisitti, Tyranni, and Passeri) according to their evolutionary history. Among these, the largest is the Passeri, which comprises around 5,000 species, also known as oscine passerines. They include most of the common families from sparrows (Passeridae), finches (Fringillidae) and thrushes (Turdidae), to swallows (Hirundidae), starlings (Sturnidae) and Old World warblers (Sylviidae) (Mayr 1946; Sibley and Ahlquist 1990; Gill *et al.* 2010).

The true finches (Fringillidae) are one of several lineages of granivorous passerines. They occur in Africa, Eurasia, and North and South America. The greatest diversity in terms of their number of species and genera is found in Eurasia (Sibley and Ahlquist 1990; Clement *et al.* 1993; Collar and Newton, 2010).

Ankober Serin (*Serinus ankoberensis* Ash 1979) is one of the finches first recorded in 1976 in the Ankober area (Ash and Atkins 2009; Spottiswoode *et al*. 2010). It is grouped under Order Passeriformes, Sub-Order Passeri and Family Fringillidae. Recent taxonomic revisions propose its placement under the genus *Carduelis* and more recently to the resurrected genus *Crithagra* (Zuccon *et al.* 2012). Formerly, Ash (1979) stated that *Serinus ankoberensis* (Fig 1A) is close to Yemen Serin (*Serinus menachensis,* Grant 1913) (Fig. 1B) of peninsular Arabia, which it resembles in plumage coloration. Following Hall and Moreau (1970), Ash (1979) included Ankober Serin in a species group of Streaky–headed Seedeater (*Serinus gularis,* Smith 1836) (Fig. 1C), Reichard's Seedeater *(Serinus reichardi*, Reichenow 1882) (Fig. 1D), Black-eared Seedeater (*Serinus mennelli,* Chubb 1908) (Fig. 1E), Streaky Seedeater (*Serinus tristriatus*, Rüppell 1840) (Fig. 1F) which are plain-coloured and streaked serins in one plumage or another, lacking any bright rump coloration. Both *S. ankoberensis* and *S. menachensis* have pointed wings. However, unlike *S. menachensis*, which has relatively conical bill, it has pointed bill (Ash and Atkins 2009; Spottiswoode *et al*. 2010).

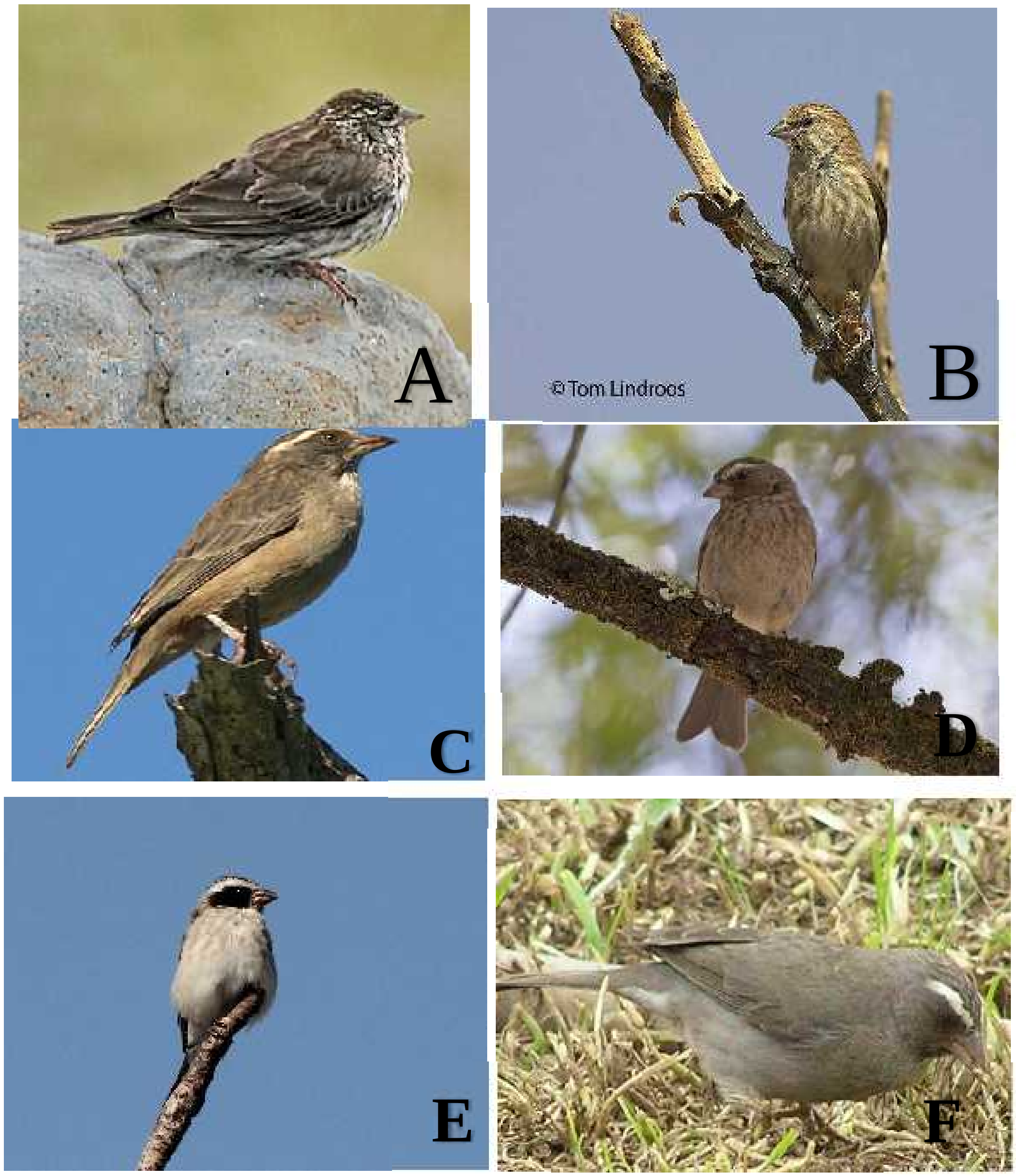


Figure 1. (A) Ankober Serin (*Serinus ankoberensis*), (B) Yemen Serin (*Serinus menachensis*), (C) Streaky–headed Seedeater (*Serinus gularis*), (D) Reichard's Seedeater (*Serinus reichardi*) (E) black-eared seedeater (*Serinus mennelli*) *and* (F) Streaky Seedeater (*Serinus tristriatusi*) *(*Source: Wikipedia https:// en.m.wikipedia.org)

Though Dowsett and Forbes-Watson (1993) have included *S. ankoberensis* as a subpsecies of *S. menachensis*, the two species are retained as separate (Sibley and Monroe 1990, 1993). Based on this view, the taxonomic scheme of Ankober Serin is shown below (Fig. 2).

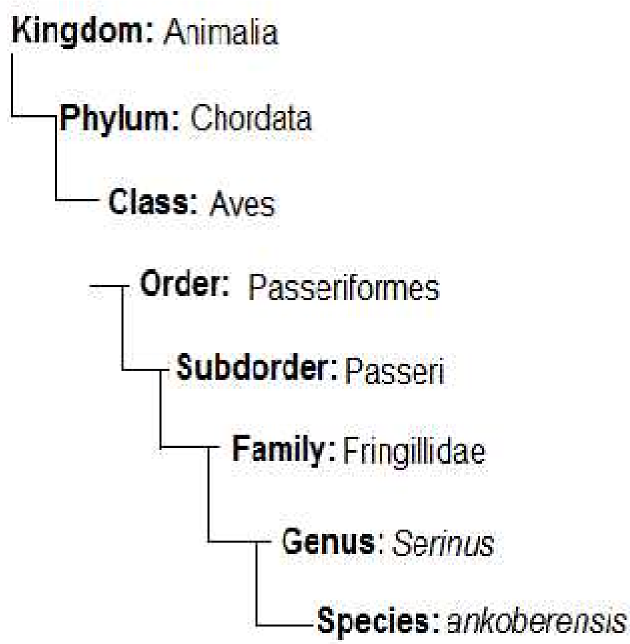


Figure 2.The taxonomic scheme of Ankober Serin (Source: Sibley and Monroe 1990, 1993).

However, based on mitochondrial and nuclear DNA sequences, Zuccon *et al.* (2012) have proposed that the genus *Serinus* was polyphyletic and thus split in to a number of species including the Ankober Serin that was moved to the resurrected genus *Crithagra*. Ankober Serin was not included in their analysis of comparison of the topologies. This is an indication that the species was not a subject of molecular analysis.

1.1.2. Flocking and feeding behavior

Ankober Serin is primarily a seed eater (Vivero 2004). It feeds on grass seeds most commonly in flocks and rarely individually. It is sociable, two or three birds feed together in close bodily contact. Ankober Serin is a gregarious species occurring in flocks of 50–100 individuals (Anteneh Shimelis 1999). The flock usually rise in a compactly and circle round high in buoyant flight before returning to feed (Ash and Atkins 2009). Flocking is said to be important for saving foraging time and improving the efficiency of foraging (Beauchamp, 2005; Michelena and Deneubourg 2011). Food abundance and risk of predation are key factors leading birds into flocking (Conradt 1998; Heithaus 2005; Luo *et al*. 2012; Beauchamp 2013). By increasing flock size, birds decrease their vigilance effort and decrease their risk of predation. This helps them to gain energy by increasing their feeding time (Michelena and Deneubourg 2011).

1.1.3. Morphology and voice

Ankober Serin is a type of finch with body size of 11–12 cm (see also table 1). The pelage is dorsally grey brown with dark brown streaks more pronounced on the head. It is also streaked from throat to vent. The bill is end-pointed. The streaky seedeater *Serinus striolatus,* a similar species is very much larger, with much larger bill (Birdlife International 2012).

The holotype in Ash (1979) had brown streaked paler mantle, paler brown lower back, streaked darker rump, paler brown upper tail coverts, darker and broadly streaked head, brown ear coverts, indistinct moustachial streak, whitish-grey chin, uniformly dark brown wings, dark brown iris, near dark neutral gray mandible horn (the lower much paler) and brown tinged flesh tarsus. He also noted that the description of the holotype is very close to his other Ankober Serin samples he collected. For the features described above, see figures 3. A, B, and C.

As Ash (1979) has remarked, no major differences could be seen between any of these birds and the breeding pairs. Thus, sexes are not easily distinguishable. Slight individual variation was seen on the facial pattern of the birds. Some showed poorly defined moustachial streak and paler cheeks; which could be males. These characters were observed on the singing male but not on the female.

Ankober Serin has soft, nasal szhree contact call and high-pitched, trilling flight call (Birdlife International 2012). A musical chirruping is heard when the flock gathers on cliffs (Ash 1979).

Table 1. Measurements on Ankober Serin (Source: Ash 1979)

Specimen	**Body length (mm)**	**Wing length (mm)**	**Tail (mm)**	**Tarsus (mm)**	**Weight (g)**
Holotype	125	75	51	16	15·1
First year male	120	74	51·5	15·8	14·6
Unsexed bird	130	74	53	16·5	15·0

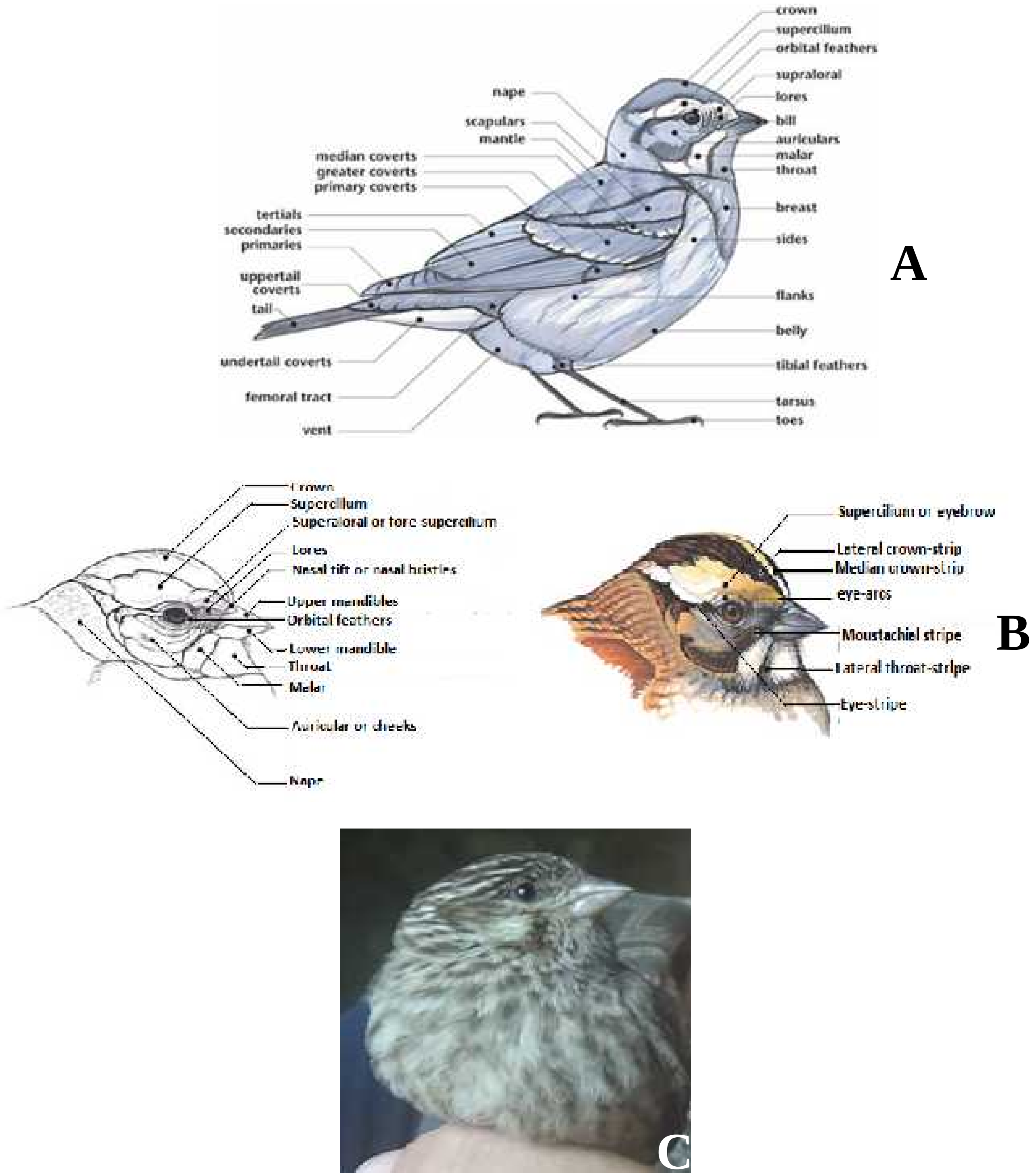


Figure 3. Topography of a passerine for reference of external features (A) and (B) and photo of Ankober Serin from GCCA (C) (Source: Sibley 2009; photo: Abebayehu Desalegn 2015).

1.1.4. Breeding and clutch size

Ankober Serin breeds at elevations of about 3030 m along broken cliff tops (Ash 1979). As noted in EWNHS (1996), breeding takes place between October and March, although it breeds during any season following heavy rains. The clutch size was observed to be normally three, which is laid in overhanging nests constructed in vertical holes of cracked surfaces (EWNHS 1996; Ash and Atkins 2009).

1.1.5. Habitat and distribution of Ankober Serin

Ankober Serin inhabits along the escarpment rim of the Ethiopian highlands in open terrain that includes broken hill-tops, near-vertical cliffs, steep, vegetated slopes and earth banks (EWNHS 1996; Anteneh Shimelis 1999). It prefers to perch on lichen-covered rocks, bare earth and short-grazed pasture, ploughed land and feeds on seeds of grasses and herbs (EWNHS 1996; Vivero 2003). Ash (1979) has noted that Ankober Serin lives and breeds at around 3030 m (10 000 feet) along broken clifftops in Ankober area, where it was discovered. In the Ankober area the cliffs are with pieces of near vertical rockfaces having little interspersed vegetation. Stunted *Erica arborea* was dominant in the breeding area. In places where the birds were seen, over 40 plant species were identified, of which the common species were the dock (*Rumex neruosus*), ladies mantle (*Alchemilla* spp.), clover (*Trifolium* spp.), mallow (*Sida ternate*), tree heath (*Erica arborea*), creeping red (*Thymus serpyllum*), *Hebenstretia dentata*, pincushion flower (*Scabiosa columbaria*), everlasting flower(Helichrysum spp.), fairy tails (*Pennisetum* spp.) and blue stem (*Andropogon* spp). It is also reported that Ankober Serin prefers rocky and open habitats located on steep slopes with vegetation characterized by alpine moorland, Juniperus trees and olive forest. Its habitats are commonly highlands above 2600m a.s.l.

(Spottiswoode *et al.* 2010). The bird spends most of its time on rock faces, usually clinging to vertical stone faces and bare earth banks. Almost exclusively, it alights on grass than any other vegetation (Ash 1979). Ankober Serin is endemic to the highlands of central and northern Ethiopia (Fig 4). In 1997, a flock of more than 50 individuals were seen and photographed per visit in Chennek Camp and Bhawit in Simien Mountains National Park, at altitude ranges between 3,500 m and 4,250 m (Anon 1997; Anteneh shimelis 1999; In 1989, 60 individuals were seen at one site near Ankober, Shoa province, eastern Ethiopia at elevations between 2,800 and 3,750 m (EWNHS 1996); in 1991, 20-40 were seen at another site inside the top of a ravine south of Debre Sina at 3,250 m elevation; and 13 birds were seen in Deneba and >100 birds during a two-week survey in Koreta, a very small area within Guassa Reserve (Anteneh shimelis 1999). In 2002, 300 birds were found in three days between the altitude ranges 2,800−4,300 m in the Abuna Yosef Mountains (Vivero 2003). It was noted that the species may occur in all ecologically similar habitats throughout the highland massif of Amhara Regional State and parts of Tigray, and all along the eastern mountain escarpments from Ankober to Simien, including Abuye Meda, Amba Farit, and Mt. Guna, and perhaps in Choke Mountains (Vivero 2003) (Fig 4).

1.1.6. The status and threats of Ankober Serin

The conservation status of Ankober Serin in the IUCN Red List is Vulnerable and the main threat is habitat loss due to agricultural expansion (BirdLife International 2012). Based on previously published Red List assessments, the species was Endangered in 1994 and in 1996, Threatened in 1988, again Endangered in 2000 and Vulnerable (VU) between 2004–2008. According to BirdLife International (2012), the species requires downlisting to Near Threatened if it is found to be very tolerant of habitat alterations occuring within its range.

Though Ankober Serin‘s habitat is well protected naturally due to the steepness of the terrain, the habitat in the Ankober area is under pressure due to habitat encroachment (grazing and cultivation of new lands) that resulted from human and livestock population increase. *Eucalyptus* plantation also creats a serious problem in the Ankober area (EWNHS 1996; Vivero 2001). These factors contribute to the fragmentation of the habitat of Ankober Serin.

Figure 4. Distribution map of Ankober Serin in northern (A) and central (B) highlands of Ethiopia (Source: Birdlife international 2017).

1.2. Litrature review

1.2.1. Diversity and distribution of birds of Ethiopia

The diverse habitat types of Ethiopia contribute for the tremendously diverse avifauna. According to Vivero (2001), Ethiopia and Eritrea together form one of Africa's birding hotspots possessing 861 species of birds, including 30 endemic species, representing 3.3% of the total avifauna between the two countries. This diversity and high level of endemism is attributed to the varied topographic features of these countries. The endemic species of Ethiopia are 16, while the species restricted to the geographical region of Ethiopian Highlands and shared with Eritrea are 14. Lepag (2013) on the other hand, indicated that Ethiopia harbours 837 bird species, 18 of which are endemic and a further 14 are near endemic (shared with Eritrea). More recently, the record of bird species in Ethiopia is 924 (Wikipedia https:// en.m.wikipedia.org).

According to the Ethiopian Wildlife and Natural History Society (EWNHS) (1996) and Ash and Atkins (2009), birds of Ethiopia are grouped into the following fourbiome assemblages: (1) the sahel with its 16 species; (2) The Afrotropical Highland Biome Species that includes 49 species with seven endemic; Bale Mountains National Park represents over 80% of this assemblage; (3) The Somali-Massai Biome Species with over 98 species of which six are endemic. This biome is noted as the richest; (4) The Sudan-Guinea Savannah Biome Species composed of about 16 species. Gambella is the richest area for this biome assembledge and it is the least known of the three biomes.

To promote conservation of these birds and their habitats, 69 Important Bird Areas (IBAs) were identified in Ethiopia (Fishpool and Evans 2001). The present study areas Semien Mountains National Park (SMNP) and Guassa Community Conservation Area (GCCA) are parts of the IBAs of Ethiopia.

1.2.2. Population status of some Ethiopian endemic bird species

About 38% of the Ethiopian endemic species of birds are listed as vulnerable (Vu), while 37% are least concern (LC), 19% Endangered (EN) and 6% near threatened (NT) (Vivero 2001) (Appendix 1). Population size of a number of species in Ethiopia is declining. For instance, the Endangered Ethiopian Bush Crow (*Zavattariornis stresemanni*), the Vulnerable Prince Ruspoli's Turaco (*Tauraco ruspolii*), the Critically Endangered Liben Lark (*Heteromirafra sidamoensis*) and the Vulnerable White-tailed Swallow (*Hirundo megaensis*) which are all endemic and range restricted to south Ethiopia have been declining due to shrinkage of their habitat ranges (Borghesio and Giannetti 2005; BirdLife International 2012).

1.2.3. Bird population census

Regular bird surveys help to track bird population changes in their dynamic habitats. In general, smaller population size is associated with greater risk of extinction locally, regionally, or globally. Such information is collected by undertaking surveys over varying geographical areas. The lists of globally threatened bird species or species of conservation concern in individual continents, countries or regions are based largely on information on population size (Birdlife International 2000). Bird survey data can be used to assess adverse impacts of the development activities in diverse habitats (Carter *et al*. 2000; Gregory *et al*. 2002).

1.2.3.1. Bird population census methods

Some of the methods used in bird population census in the field include; Mapping, transects (line transects and point transects), counting roosts and flocks, counting leks , counting migrants, capture techniques, tape play back and vocal individuality.(Sutherland *et al.* 2004; Sutherland 2006). Of these, the most commonly used methods are mapping, line and point transects and flock count which are further described below.

Mapping

Mapping (also called territory mapping) is used for territorial breeding species, e.g. some ducks, game birds and raptors, and most temperate passerines. In this method, the breeding territory is used as a census unit. Thus, it becomes possible to identify clusters of sightings and to estimate directly the total number of pairs or territories of each species present (Sutherland *et al.* 2004; Sutherland 2006). Many bird species in temperate areas are highly territorial during breeding seasons and males sing to defend territories.

Mapping is widely used but it is very time-consuming and requires high quality maps of the study area. Although it has such drawbacks, it is useful in generating a detailed map of the distribution and size of territories (Marchant *et al*. 1990; Gibbons *et al*. 1996; Bibby *et al*. 2000). Mapping methods combined with nest finding, radio telemetry and mist netting can also be more useful (Sutherland *et al.* 2004).

Transects

There are two types of transect methods. These are line transects and point counts (or point transects). Both are based on recording birds along a predefined route within a predefined survey unit. In the case of line transects, bird recording occurs continually, whereas for point transects it occurs at regular intervals along the route and for a given duration at each point. Both are highly adaptable and can be used in terrestrial and marine systems. They can be used to survey individual species, or groups of species. They are efficient in terms of the quantity of data collected per unit of effort expended, and for this reason they are particularly suited to monitoring projects. Transects can also be more helpful if combined with other count methods such as sound recording, mist netting and playback (Whitman *et al*. 1997; Haselmayer and Quinn 2000).

Counting flocks

Flock count is used for flocking species, particularly waders, wildfowl and some passerines. Counting flocks of large birds is easier than counting smaller birds at greater distances. This method is mostly used outside the breeding season. When searching an area systematically for flocks the data obtained can be markedly non-normal in distribution (e.g. many areas with no birds, and thus zero counts, and a few areas with very large counts); such data can present problems during statistical analyses. In order to avoid over estimation of sizes of small flocks and underestimate size of large flocks, repeated counts of a flock and involving more researches to count the same flock is useful (Sutherland 2004, Sutherland 2006).

This method is recommended for bird species which form small to moderate flock size of up to 500 individuals (Gregory et al. 2004). The method is a type of total count, which is also appropriate for species like Ankober Serin that inhabit open habitats with high chance of detection (Sutherland 2006).

1.2.4. Habitat disturbances and level of encroachment

Understanding factors affecting the distribution and abundance of animals is crucial for habitat management. Human intervention and intensified grazing by livestock are main factors that cause habitat degradation. Grazing, however, is important in certain habitats to prevent vegetation succession and maintain suitable vegetation structure for animals. Grazing, on the other hand, encourages unpalatable and low growing plants that can tolerate repeated defoliation, particularly grasses and rosette forming species. Short and medium term grazing can be used to influence the structure and composition of habitats. But, long term grazing and browsing prevent plants from regeneration and there by affect the composition of plants (Sutherland *et al* 2004; Newton 2013).

Persistent human and livestock disturbances could affect the population levels of birds by excluding birds from fully using their resources in their habitat and reducing the total habitat and food available to them. Such persisting disturbsnces mainly affect during cold weather when birds struggle to obtain enough food, as it was seen in Yellow hammers (*Emberiza citronella,* Linnaeus 1758). Yellow hammers prefer feeding over vigilance during the short winter periods (Cresstwell 2008).

It was also suggested that over-grazing is a possible source of desturbance for many farmland birds, although, the direct impact of grazing on birds is poorly known. Disturbances mainly during laying or incubation may cause many species of birds to leave their areas (Sutherland *et al* 2004; Newton 2013).

Comparing animal distributions between sites with differing levels of disturbances is an approach used to measure the effects of habitat disturbances. Studying disturbance was necessary to relate the level of disturbance to the ecology of the species (Sutherland *et al* 2004).

1.2.5. Habitat analysis

Habitat associations relate to the distribution data of species, such as the presence, abundance, or nest site location. Using wide and different sites for area comparisons are more likely to reveal the habitat association of a species. This approach can be carried out on a range of scales. At a patch scale, the frequency with which different foraging patches are used could be quantified and related to habitat, while at a site scale, the density of birds in different forest blocks or on different lakes could be related to habitat. Habitat choices determine the acquisition of critical resources such as food (MacArthur *et al.* 1966; Cody 1974; Willson 1974; Rotenberry and Wiens 1998) and refugia from predators (Leber 1985; Martin 1988; Heithaus and Dill 2002; Eggers *et al*. 2005), which in turn influence fitness and demography of the species concerned.

Useful measurements of vegetation as habitat for birds can be obtained by systemstically recording all plant species present in quadrats although sometimes certain plant species are critically important for nesting or providing fruits or seeds as food. It is usually difficult to relate the bird abundance to the abundances of a long list of plant species (Rotenberry 1985). In practice the habitat structure is usually more important than species composition. Bird habitat studies usually involve more rapid measures of the cover of dominant species and broad taxonomic groups or morphospecies (e.g. grasses) or plant species of higher taxa that are known to provide focal birds with important resources that are specific to them, such as palatable leaves, seeds, and nest sites (Sutherland *et al.* 2004).

The type and structure of vegetation provide nest sites, roosting locations, refuge from predators, and food for herbivorous birds or a stock of bird prey for flesh eating birds. The structure also enables or constrains foraging (Sutherland *et al.* 2004). Studies of small quadrats over the entire area are not practical. Thus, studies of a few representative samples of particular habitat types can be made to identify their defining characteristics, such as tree density or species composition. The height, structure, and density of vegetation often affects birds by providing perches or cover and by limiting the bird's field of view and ability to run or fly to capture prey (Sutherland *et al.* 2004; Sutherland 2006).

1.2.6. Threats to birds and their habitats

The major threat to song birds, to which Ankober Serin is grouped, is the fragmentation of habitats as it increases the vulnerability to predators and parasites (Garrett, 2008). Due to open access of local people to resources for fuel wood, conversion of land for permanent agriculture, loss of biodiversity especially loss of flora that in turn directly affects fauna of the area has become the major problem of Ethiopia and elsewhere developing Africa (Vivero, 2001).

The population status of endangered and vulnerable endemic bird species of Ethiopia is small. For example, the estimate of the endangered Ethiopian bush crow (*Zavattariornis stresemanni*) was 2,500–9,999 mature individuals (BirdLife International, 2012). Prince Ruspoli's Turaco (*Tauraco ruspolii*) was placed in the band of 2,500–9,999 mature individuals (BirdLife International 2012). The 'Critically Endangered' Liben Lark (*Heteromirafra sidamoensis*) had only 230–523 individuals (Collar *et al*. 2008). The 'Vulneable' White-tailed Swallow **(**Hirundo megaensis) was estimated to be between 3000 and 10,000 individuals (BirdLife International 2015). The main threats to these Ethiopian vulnerable and endangered bird species are habitat degradation due to agricultural expansion and overgrazing following increased human population and cattle densities, uncontrolled bushfires, illegal logging and destruction of nesting sites (Borghesio *et al.* 2004; Coppock 1994; Ayana Angassa and Fekadu Beyene 2003; Homann 2004; Dalle *et al.* 2006; Solomon *et al.* 2007; Borghesio and Giannetti 2005; Mellanby *et al.* 2008 and Donald *et al.* 2012). Limited habitat range with a fixed range of temperature and drought, scrub encroachement, large mines, illegal capture of birds and collection of eggs for the bird trade and medicinal purposes in some parts of Ethiopia are also considered as increasing threats (Borghesio *et al.* 2004).

African Wildlife Foundation (AWF) (2015) stated the major cause for ecological degradation and loss of biodiversity in SMNP is the increase in human population and unsustainable use of natural resources by the local people. More than 67% of the park has been used by the local communities for grazing, subsistence agriculture and settlements (AWF 2015). Competition for grazing between domestic animals and the wild herbivores, transmission of disease from domestic to wild animals and *vice-versa*, and human–wildlife conflicts that intensify ecological degredation and habitat fragmentation in the area are apparent (UNESCO/IUCN 2006).

1.2.7. Conservation of bird habitats in Ethiopia

Some of the systems that contributed to the conservation of bird habitats in Ethiopia are the traditional conservation systems called “Qero” system and “Geda” management system. “Qero” system was a communal system in Guassa area. Guassa is named after the *Festuca* grass. The“Qero” system protected the *Festuca* grassland ecosystem for more than 400 years (Zelalem Tefera and Leafder-Williams 2006). The “Geda” system is proved to be effective in conserving habitats of the bush-crow (Bassi and Tache 2011). “Geda” (Gadda) is the traditional social stratification system of Oromos in Ethiopia. This system practices moving livestock from one area to another depending on the season and bush fire to control bush and scrub encroachment (Gemedo *et al.* 2006). Gazzetment of protected areas and redemarcation of their boundaries as in the SMNP and GCCA to include the suitable habitat of the birds and other endemic animals is being practiced in Ethiopia.

In general, bird conservation actions in Ethiopia are not effective due to various reasons. Mellanby *et al.* (2008) pointed out that there is no active management and no enforcement of regulations in some protected areas. They also added that responsibilty for protected areas has now been passed to the Regional Governments. Thus, collaboration between Regional and Federal Governments and local communities is required to achieve more successful protection of wildlife and their habitats (BirdLife International 2015).

Some of the conservation measures suggested to secure population recovery and stability of the endangered and vulnerable endemic species in Ethiopia are: (1) sustainable use of resources without affecting bird habitats and with no exclusion of people and their grazers; (2) monitoring bird population changes; (3) encouraging traditional management systems such as Geda and Qero; (4) retention of roosting and nesting habitats; (5) controlling expansion of agriculture; and (6) initiating gazzetment of protected areas for active management and redefine the boundary to include the suitable habitat of birds (BirdLife International, 2015).

1.2.8. Diet composition

Wide range of ecological questions related to what a species forages on and why the species prefers certain habitats over other habitat types can be answered by studying the diet and foraging behavior of a given species (Sutherland *et al.* 2004). Most grassland species are generalized omnivores. They feed on insects and seeds. In breeding seasons, insects are taken by the birds in higher proportion mainly to feed the nestlings. The food resources in grassland birds are the products of a variable climate and vary between years and locations (Cody 1985).

Resource tracking is an ecological response where species maintain the behavioral flexibility to find out and utilize sites where resources are sufficiently available (Cody 1985).

Insectivorous passerines are by far the most numerous of all birds (Moreau 1972; Klasing 2000; Asokan *et al.* 2009). This success is believed to be due to the fact that the nutritional value of insect diet is high. Insect diet is rich in easily digestible protein and fat (Morse

1975; Klasing 2000; Nakano *et al.* 2007; Dorn *et al.* 2011; Buij *et al*. 2012). Most granivorous, frugivorous, nectarivorous and herbivorous birds feed their young a diet of insects, spiders, and other invertebrates. Some birds are highly insectivorous during certain parts of the year depending on species, availability and environmental conditions. Insect diet is highly nutritional.This is seen in the winter food of a small insectivorous passerine, golden-crowned kinglet (*Regulus satrapa*) (Heinrich and Bell 1995). This bird is among the smallest of birds (weighing only 5 g) that winter in an environment that routinely drops to -30°C feeding almost exclusively on hibernating insect larvae. The Ethiopian bush-crow‘s diet also includes flying and crawling insects, caterpillars, termites, grasshoppers particularly beetles and maggots that are associated with cattle and cowpats (Benson 1946; Ash and Gullick 1989; Fry *et al.* 2000).

1.2.8.1. Methods of studying diet composition of birds

Direct observation, nest observation, remains and signs, dropping analysis, pellet analysis, stomach analysis, direct observation of crop regurgitates, neck ligatures emetics and flushing are some of the commonnly used methods to study the diet composition of birds (Mary 2000; Sutherland 2004). Some of these methods are discussed below:

Direct observation

Direct observation enables to obtain useful information when birds feed. Such information is especially useful when combined with an examination of the range of prey available. Direct observation is particularly useful for seed-eating birds that remove the hard seed coat (testa) before eating the seed and birds that feed on invertebrates with few hard parts. In most cases, observation of feeding methods can narrow the range of possible items but other techniques are necessary to fully determine the diet. Direct observation is usually limited to species that occupy open habitats (grassland, savanna, or tundra) and forage during daylight periods. Observations in nests also have provided information on foods brought to feed juveniles (Errington 1932; Marti 1987; Bielefedt *et al.* 1992). Foraging adults often swallow small items they find, but take large prey back to the nest. Watching from a hide overlooking the nest can be useful. Reliable data on the diet of chicks can be obtained using nest cameras with an infrared beam fixed so the bird triggers the camera as it returns to the nest. For birds using nest boxes, the camera can be placed inside so that entering birds are photographed (Mary 2000; Sutherland 2004).

Food remains and signs

Food remains and signs can be identified when birds leave evidence of food items they have taken. For herbivorous species bite marks on vegetation give useful clue, while for seed-eaters look for discarded husks are good indicators of the diet. The remains of vertebrates that are not eaten in whole can be examined to determine species, age, sex, condition, or parasite load (Mary 2000; Sutherland 2004).

Dropping analysis

Dropping analysis is used to examine hard remains in the fecal samples (a mixture of feces and urine). Droppings can be collected from birds caught for ringing. This is easiest if the birds are held in clean bird bags. Droppings can also be collected in the field. In some cases, such as under roosts, nests, or perches, one can be confident which species or even which individual produced which dropping. The method is non-destructive and large samples can be collected (Mary 2000; Sutherland 2004).

Pellet analysis

Pellet analysis is used to quantify the diets of birds such as owls which regurgitate pellets comprising bones and other hard parts along with fur and feathers. In dropping and pellet analysis, differential digestibility limits evaluation of importance of various foods (Mary 2000; Sutherland 2004).

Stomach content analysis

Stomach content analysis can be used if birds are found dead and if there is undigested food in the stomach. The stomach is removed as soon as possible and placed in high concentration of alcohol in order to prevent the deterioration of the contents. Shooting birds solely to determine diet is nowadays usually considered unacceptable (Rosenberg and Cooper 1990, Mary 2000; Sutherland 2004).

1.2.9. Study of genetic diversity

Successful conservation programs need to be based on knowledge of the genetic diversity of the study species. This knowledge helps to define management components required to minimize the loss of genetic diversity and to preserve the existing genetic structure of the species (Haig 1998; Hedrick 2001). Information about taxonomic status of the species, the spatial limits of populations and the nature of gene flow among populations can be obtained through population genetic analyses (Pérez de Rosas *et al.* 2007; Fitzpatrick *et al.* 2008).

1.2.9.1. The use of molecuar markers

Molecular markers are powerful tools for the analysis of genetic diversity of organisms. They allow indirect estimates of many ecologically important parameters, and if available, would greatly facilitate conservation and management of species. Of the various types of molecular markers, microsatellites have many positive attributes, including hypervariability, co-dominance, abundance and tolerance to variation in DNA quality and quantity (Selkoe and Toonen 2006).

The wide range of vailable techniques such as restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) can be used to evaluate DNA polymorphism and directly measure genetic diversity. As these molecular markers show Mendelian inheritance, they can be used to trace the fingerprint of each of the organisms and determine the evolutionary history of the species by phylogenetic analysis, studies of genetic relationship, population genetic structures and genetic mapping (Hamilton *et al.* 1999; Zane *et al.* 2002; Hoshino *et al.* 2012).

Specifically, microsatellite markers are powerful for revealing inter- or intraspecific phylogenetic relationships, even in closely related species (Wang *et al.* 2009). Thus, microsatellites have been widely used as markers in studies of genetic diversity analysis in birds (Hammond *et al*. 2002; Aowphol *et al*. 2008; Jamieson 2009). Microsatellites occur in all prokaryotic and eukaryotic genomes and they are defined as tandemly repeated motifs of 1–6 bases (usually di-, tri-, or tetranucleotide). In eukaryotes, they are generally identified in large blocks of non-coding segments of the heterochrotic region, which is concentrated towards the centromere and differentiated from the euchromatic regions away from the centromere (Hoshino *et al.* 2012).

Microsatellite analysis is used to record relative sizes of the duplicating segments. Each locus is categorized electrophoretically, separating labeled microsatellite PCR fragments and measuring the relative size (in base pairs). The labelled fragment analysis is, therefore, based upon the same principle of analyzing varied length polymorphisms. The basic principle involves the labeling of a DNA fragment with dye markers (traditionally labeled by radioactive tags) during PCR, followed by electrophoresis, fluorescence reporting, and size estimation using labeled standards. The CEQ8000 genetic analyser has a laser that excites the dye label to fluoresce just as the varied length amplicons electrophoretically pass through a capillary detection window at rates proportional to their size (Zane *et al.* 2002; Dewoody *et al.* 2006; Amos *et al.* 2007). Figure 5 shows microsatellite profile for an individual after running on the CEQ8000. Depending on the type of marker, such profiles form the characteristic of the locus under investigation (Zane et *al.* 2002).

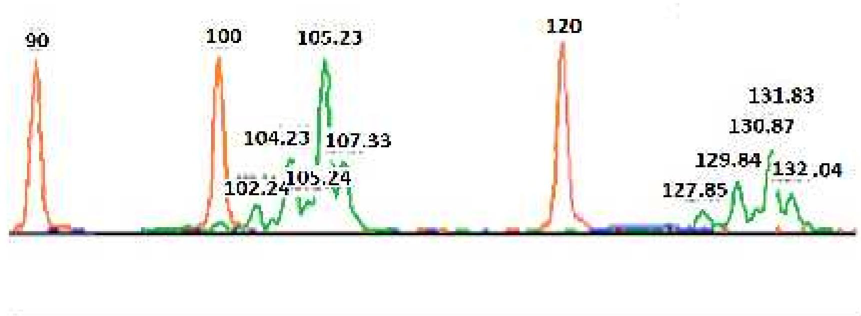


Figure 5. Microsatellite profile of an individual with the numbers indicating number of nucleotides (Source: Zane *et al.* 2002)

Few studies on the genetic structure of Ethiopian birds have been undertaken using techniques other than microsatellite analysis. For instance, genetic study together with morphological and vocal evidences of the endangered populations of the Liben Lark (*H. sidamoensis*) of southern Ethiopia and the Archer's Lark (*H. archeri*) in north-east of Ethiopia and Somaliland that are separated by 590 km, in the Horn of Africa, was carried out using mitochondrial cytochrome b (cytb) gene (Spottiswoode 2013). A 308 bp segment of the mitochondrial *cyt b* region was also used to determine the taxonomic status of the Degodi Lark (*Mirafra degodiensis*) (Collar *et al.* 2009).

1.2.9.2. Genetic differentiation and gene flow

Due to their ability to fly, birds can move over large geographical scales and their populations are therefore often spatially more homogeneous than populations of other taxonomic groups (Barrowclough 1983; Evans 1987; Ward *et al.* 1992). Thus, early surveys of wild birds led to the general perception that genetic differentiation is lower in birds than in other vertebrates at both the population and species levels (Barrowclough and Corbin 1978; Avise and Aquadro 1982; Barrowclough 1983). There are some exceptions from this general pattern. For example, Senar *et al.* (2006) found two populations of the highly mobile Citril finch (*Serinus citrinella*) to be significantly differentiated in the presence of gene flow, even though their breeding areas were separated by only 5 km and the two populations are mixed in a common overwintering area in Spanish Pyrenees. Two non-mutually exclusive factors were imagined for this differentiation. One was selection acting on individuals with the 'wrong' genotype; with the assumption that there is a connection between the diversity at the microsatellite level and phenotype. The other was the phenotype-dependent dispersal, so that individuals facing the wrong environment are more likely to disperse than others, which then might act to promote the differences. This assumes genetic differences at a large scale and not only in a few microsatellite loci.

Significant genetic differentiation was found also among migratory Swainson's warbler (*Limnothlypis swainsonii*), populations that are 435 km apart and with no steep environmental gradients or obvious barriers to gene flow between southern Louisiana and eastern Arkansas that would promote differentiation. It was explained that, due to lack of obvious barriers to dispersal between Arkansas and the coastal plain, vaicariance events on the breeding range, a split wintering range, or both could contribute to the pattern of differentiation observed (Winker *et al.* 2000).

Genetic studies on the Finnish or Stockholm house sparrow (*Passer domesticus*) populations that showed significant genetic differentiation was attributed to the open sea between Finland and Sweden which forms a dispersal barrier for these species. The distance of 250 km between Turku (Finland) and Stockholm (Sweden), including approximately 40 km of open sea, was sufficient to create significant genetic differentiation among the populations (Kekkonen *et al.* 2011).

Though the Horn of Africa populations, Liben (Sidamo) Lark (*Hetromirafra sidamoensis*) and Archer's Lark (*H. archeri*) are conspecific, both were distinct from Rudd's Lark (*Hetrmirafra Ruddi*) in South Africa which is also attributed to the enormous gap in the distribution (Spottiswoode 2013).

Low levels of genetic differentiation were found within the Finnish house sparrow in a contiguous landscape. This low genetic differentiation between the Finnish house sparrow populations was probably attributed to gene flow over large geographical distances (longest distance between sites was 813 km) by a stepping-stone pattern of small migration distances in a fairly homogenously distributed population. Additionally, the colonization history of the house sparrow to Fennoscandia after the last ice age was suggested for the low genetic differentiation between the house sparrow populations (Kekkonen *et al.* 2011). Similarly, the interpopulation distances among Finnish and Swedish willow tit (*Poecile montana*) populations is up to 1000 km. However, Kvist *et al.* (1998) found no genetic differentiation among these populations. This was also suggested to be due to high gene flow across relatively homogenous landscape lacking geographical barriers.

Molecular studies on the Liben (Sidamo) Lark and Archer's Lark showed that Archer's Lark from Somali land and Liben Lark from the Liben Plain and from Jijiga (Ethiopia) were not only genetically very similar but did not even form separate monophyletic groups. This is suggestive of recent gene flow between the Horn of Africa populations, or very recent separation (Spottiswoode *et al.* 2013).

1.2.9.3. Factors affecting genetic structure of populations

Genetic studies on avian populations revealed that sedentariness, small population size, island-like distribution, and stable range history (e.g., lack of glaciation) are important factors promoting differentiation (Bossart and Pashley 1998). There is the tendency for tropical birds to have higher levels of differentiation than temperate ones at both the population and species levels (Braun and Parker 1985; Capparella 1988; Zink 1988; Hackett and Rosenberg 1990; Capparella 1991; Peterson *et al.* 1992; Hackett 1993; Seutin *et al.* 1993; 1994; Bates and Zink 1994; Brumfield *et al.* 1997). For example, allozyme-based studies suggested that, on average, populations of Neotropical bird species exhibit levels of differentiation several-fold higher than those found in North Temperate birds. Latitudinal differences in climate may have been important factors in producing apparent difference between Neotropical and North Temperate birds. Holarctic regions experience greater longterm climatic fluctuations than tropical regions because seasonality correlates with latitude (Cuffey *et al.* 1995; MacAyeal 1995; Thompson *et al.* 1995). Annual and longterm climatic fluctuations may have had profound homogenizing effects on the genetics of many North Temperate bird populations. Therefore, longterm phenomena such as glaciations and shortterm phenomenon of climatic seasonality may have significant effects on population structure and gene flow.

Longterm phenomena such as glaciations can cause dramatic fluctuations in the size and shape of a species' geographic range. This, in turn, affects the size of populations, their degree of isolation, and the residency times during which they may undergo differentiation. Longterm stability is likely to be an important factor favoring differentiation among populations simply by allowing time for its development through genetic drift or local adaptation. For instance, Hammond *et al.* (2002) studied Genetic diversity in Wild and Farmed Emus (*Dromaius novaehollandiae*) using microsatellite analysis; and did not detect any genetic differences between emus sampled from wild populations that were separated by vermin-proof fences; and they reasoned out that, though the fences may be effective in preventing emu movement, the fences were erected only 100 years ago, which may be an insufficient time for genetic differences to develop. The modification of geographic ranges may create opportunities for gene flow or colonization that inhibit population differentiation. For example, several north temperate vertebrates show lower levels of genetic variation and/or population structure in formerly glaciated portions of their ranges, including salamanders (Highton and Webster 1976), fish (Bernatchez and Wüson 1998; Sage and Wolff 1986), and five bird species (Väisänen and Lehväslaiho

1984; Gill *et al.* 1993; Zink and Dittmann 1993; Merilä *et al.* 1996). Shortterm phenomenon of climatic seasonality increases with latitude, promoting seasonal migration in a higher proportion of the breeding avifauna (Newton and Dale 1996). Effective dispersal distances are greater in migratory birds, leading to higher levels of gene flow and lower levels of differentiation in north temperate bird populations (Winker *et al.* 2000).

1.2.9.4. Hardy-Weinberg equilibrium

Hardy-Weinberg equilibrium is maintained if the population size is too large, mating in the population is random, all individuals in the population have the same fitness, regardless of their genes, there is no gain or loss of genes due to immigration into or emigration out of the population and there is no new mutation in the population. Conditions that violate the equilibrium can lead to a change in gene frequency (Robinson 2002).

1.2.10. Knowledge gap and justification

Documented ecological study on Ankober Serin is not available. Current knowledge of the species is based on reports of field observations and preliminary surveys. It is hence important to conduct scientific research on the species to generate data for more reliable understanding of its ecology and biology. BirdLife International (2012) recommended research to be conducted on the population size and geographical distribution of the species in order to comprehensively understand its status and plan workable conservation actions. Also, the genetic structure of populations of these birds is almost unknown. The present study is expected to report the genetic variation in this species by sampling birds from two main areas (Guassa Community Conservation Area and Simien Mountains National Park) of the northern highlands of Ethiopia. The two study areas are at least 360 kms apart separated by range of mountains, valleys and lowlands

1.3. General Objective

The general objective of this study was to detrmine the flock size, understand the habitat charactristics, diet composition, and genetic diversity of Ankober Serin (*Serinus ankoberensis*) in two of its known ranges - Simen Mountains National Park (SMNP) and Guassa Community Conservation Area (GCCA).

1.4. Specific Objectives

The specific objectives of the present study were to:

- determine the flock size of Ankober Serin,
- assess level of disturbance in the habitat of Ankober Serin in SMNP and GCCA,
- study the cover, composition and stratification of the vegetation of Ankober Serin, habitat,
- identify the most important diet items of Ankober Serin, and to
- study inter and intra-population genetic diversity of Ankober Serin, using microsattelite markers.

1.5. Research Questions

1. What is the flock of size of Ankober Serin,at GCCA and SMNP?
2. To what extent is the level of human and livestock encroachment in the habitat of Ankober Serin?
3. What are the vegetation characteristics of the preferred habitats of Ankober Serin?
4. What are the main diet items of Ankober Serin?
5. What is the existing inter and intra-genetic diversity of Ankober Serin?

2. The study areas and methods

2.1. Description of the study areas

Due to limitation of finance, this study could not cover all areas in Ethiopia where Ankober Serin may possibly exist. Hence, only two areas Simien Mountains National Park (SMNP) and Guassa Community Conservation Area were included during the present study (Fig. 6). These two areas are also known representatives of the highlands harboring Ankober Serin with more reports of considerable population numbers (Vivero 2001).

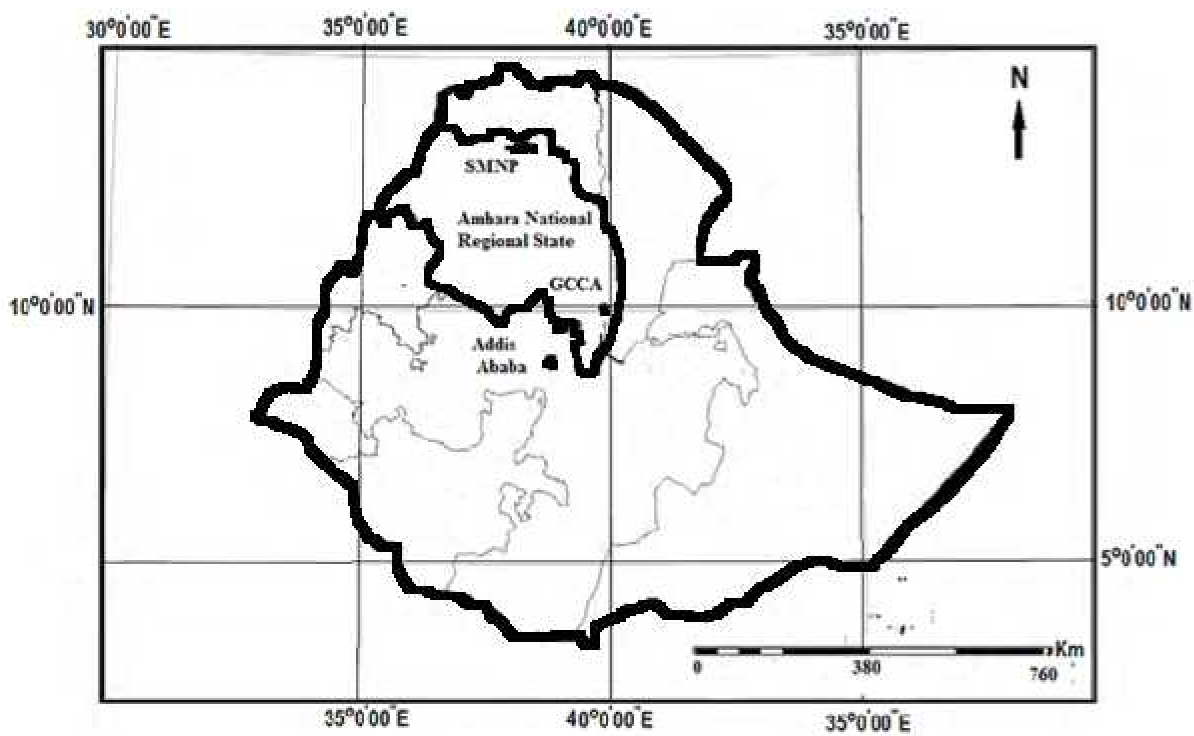


Figure 6**.** Locations of the Simien Mountains National Park (SMNP) and Guassa Community Conservation Area (GCCA).

2.2. Simien Mountains National Park (SMNP)

The Simien Mountains National Park (SMNP) is located at geographic coordinates of 13°1'–13°29' N and 37° 50'–38° 34' E, in the Amhara National Regional State, North East of Gondar town, Ethiopia. It is 886 km from Addis Ababa, the capital city of Ethiopia and 123 km from Gondar town (Fig. 7). The altitude ranges between 1900–4430 m a.s.l. The SMNP is endowed with unique landscape and endemic animals and plants that are restricted to the Ethiopian Highlands (EWCA 2013). Simien Mountains National Park has an extent of 412 km^2 (Mesele Yihune and Afework Bekele 2012; EWCA 2013). The Park was designated as a World Heritage Site in 1978 by UNESCO. The reasons for its designation as a World Heritage Site were the presence of high number of endemic species, unique bio-physical features and its international significance. In 1996, due to the declining number of Walia ibex (*Capra walie*), agricultural encroachment, and loss of biodiversity and impact of road construction the Park was enlisted as World Heritage in Danger (Falch and Keiner 2000). Currently, the wildlife status in this Park is improved because of the community based conservation activities. During the re-demarcation of the Park boundaries in 2003, the Amhara Government and communities agreed to exclude villages along the Park boundary and some areas under cultivation and to include the Mesarerya and Lemalimo Wildlife Reserves with SMNP. In 2007, it was further extended to include the peaks of Silki, Kidus Yared and also Ras Dejen (the highest mountain in Ethiopia) (Tezera Chernet 2017).

2.2.1. Topography

The Simien Mountains is characterized by high plateau, grassy plains steep escarpments, deep gorges and sharp cliffs that resulted from heavy erosions of Trappean basalt layers which had originated from lava outpourings of the Oligocene-Miocene volcanic activities (Mohr, 1962). The jagged mountain peaks, deep valleys and vertical cliffs about 1,500 m have resulted from massive erosion of the Ethiopian plateau (Magin 2001). The plateau is surrounded on the south and northeast by deep valleys of Tekeze River (Hurni and Ludi 2000). The group of mountains are separated from each other by deep valleys (Sileshi Nemomissa and Puff 2001).

The soil types of the SMNP are lithic leptosols (42%), eutric cambisols (31%) dystric podzoluviso (26%) and humic nitosols (1%) that resulted from the difference in geological formation, glaciations, topography and climate. The land use practices and the topography of the area have highly eroded the soil of the Simien Mountains (FAO/LUPRD 1982; AWF 2015).

2.2.2. Climate

The climate of SMNP depends on great altitudinal variations and the features of mountains. Rainfall is bimodal in SMNP with 1,550 mm mean annual rainfall comprising two wet seasons, from February to March, and July to September.

Simien Mountains National Park is characterized by low temperature. The temperature ranges from a minimum of -2.5°C–4°C to a maximum of 18°C. There are often dry winds during the day; frosts may occur at night, and snow sometimes settles on the summit of

Ras Dashen about elevation of 4,533 m also known as the roof of Africa (Hurni 1986; Magin 2001).

2.2.3. Vegetation

The Simen Mountains are a part of the Afro-alpine Centre of plant diversity, with high levels of endemism due to past isolation. However, the diversity is low as compared with other Afro-alpine regions due to the post-volcanic and post- glacial history (Magin 2001; Mesele Yihune and Afework Bekele 2012). Some of the plant species endemic to SMNP are the stone crop (*Rosularia simiensis*) and the tussock grass (*Festuca gilbertiana*). This Park is characterized by Ethiopian Tropical Seasonal Highland Biome having four vegetation belts -Afromontane forest, Hypericum woodland, Afromontane grassland and Afro-alpine moorland. Species in the latter two biomes show xeromorphic adaptations to extreme altitude conditions, and much speciation. However, heavy overgrazing has eroded and degraded the grassland, which is now unproductive. Out of 900 ha of Afro-alpine vegetation, 25% was heavily overgrazed, 60% heavily grazed and 15% more or less natural (Debonnet *et al.* 2006). The forest below 3,000 m is mostly destroyed except in the gorges, where some Water berry (*Syzygium guineense*), African pencil cedar (*Juniperus procera*) and African olive (*Olea europaea africana*) remain (Nievergelt 1998). Coarse tussock grasses, cliff-hanging herbs and small shrub thickets of *Rumex nervosus*, scattered *Otostegia minucci, Geranium arabicum, Thymus* spp., *Trifolium* spp., and the creepers (*Clematis simensis*) and false cleavers (*Galium spurium*) characterize the escarpment cliffs, gorge sides and ridge tops. *Erica arborea–Hypericum revolutum* (tree heather–giant St.John’s wort) heath-woodland was once the feature of the belt from 3,000 m to 3,800 m. However, this area is now with few trees remaining without regeneration as a result of clearing for growing cereals. Giant lobelia (*Lobelia rhynchopetalum)* with tree heather (*Erica arborea)*, torch lily (*Kniphofia foliosa)*, African rose (*Rosa abyssinia*), yellow primrose (*Primula verticillata* a Palaearctic species), *Solanum* spp., everlasting flower (*Helichrysum citrispinum*), lady's mantle (*Alchemilla alpine*) and *Urtica spp*. and lichens *Usnea spp*. that drape the trees characterize the area from 3,800 m to the alpine zone, which is subalpine grassland (Ashine 1982). This formerly rich and tufted mosaic of grassland has been largely replaced by short-grass turf of *Festuca macrophylla* with *Carex erythrorhiza*, and has been worn down by cattle. Mosses of the Grimmiacea family are common above the subalpine grassland, which is the alpine moorland (Ashine 1982).

2.2.4. Fauna

Including the endemic mammals such as the Gelada baboon (*Theropithecus gelada*) Walia Ibex (*Capra walie*) and the Ethiopian Wolf (*Canis simensis*), there are 22 large mammals (Table 2) (Puff and Sileshi Nemomissa 2001; EWCA 2013). These species are globally threatened and make the Park globally significant for biodiversity conservation.

Six species of rodents (*Lophuromys flavopunctatus*, *Arvicanthis abyssinicus*, *Stenocephalemys griseicauda*, *Otomys typus*, *Rhabdomys pumilio* and *Tachyoryctes splendens*) and two species of insectivores (*Crocidura baileyi* and *Crocidura thalia*) were also recorded (Mesele Yihune and Afework Bekele, 2012). Of these recoreded species *Stenocephalemys griseicauda* is endemic to the afro-alpine moorland above 3000 m a.s.l.

Table 2. Large mammals in Smien Mountains National Park (EWCA 2013)

Common Name	**Scientific Name**
Colobus monkey	*Colobus guereza*
Ethiopian wolf	*Canis simensis*
Gelada	*Theropithecus gelada*
Golden (common) jackal	*Canis aureus*
Grey duiker	*Sylvicapra grimmia*
Hamadryas baboon	*Papio hamadryas*
Klipspringer	*Oreotragus oreotragus*
Leopard	*Panthera pardus*
Menelik’s bushbuck	*Tragelaphus seriptus meneliki*
Olive baboon	*Papio Anubis*
Rock hyrax	*Procavia capensis*
Serval cat	*Felis serval*
Spotted hyena	*Crocuta crocuta*
Vervet monkey	*Ceropithecus aethiops*
Walia ibex	*Capra ibex walie*

Over 180 species of birds were recorded in the SMNP. Of these, five are endemic to Ethiopia and a further 12 to Ethiopia and Eritrea. Thirty three species represent the Afrotropical Highland Biome assemblage (Hillman 1993; EWCA 2013). Some of the birds found in SMNP are listed in Table 3.

Due to the presence of globally threatened, range restricted and highland Biome species, SMNP is selected as one of the Important Bird Areas (IBA’s) of the Amhara Region and Endemic Bird Areas (EBA’s) of the world (EWNHS, 1996). Of the endemic birds of Ethiopia, the Spot-breasted Plover (*Vanellus melanocephalus*), Blackheaded Siskin

(*Serinus nigiceps*), Abyssinian Longclaw (*Macronyx flavicollis*) and Abyssinian Woodpecker (*Dendropicos abyssinicus*) are found in SMNP. Ankober Serin is also one of the endemic species found in SMNP (Table 3) (EWCA 2013).

Table 3. List of some of the bird species found in SMNP (Source: EWCA 2013).

Common Name	Scientific Name
Abyssinian Catbird	*Parophasma galinieri*
Abyssinian Longclaw	*Macronyx flavicollis*
Abyssinian Slaty Flycatcher	*Melaenormis chocolatina*
Abyssinian/Black-headed Forest Oriole	*Oriolus monacha*
Abyssinian/Golden-backed Woodpecker	*Dendropicos abyssinicus*
Ankober serin	*Serinus ankoberensis*
Banded Barbet	*Lybius undatus*
Bearded Vulture (bone crusher)	*Gypaetus barbatus*
Black-headed Siskin	*Serinus nigriceps*
Black-winged Lovebird	*Agapornis taranta*
Red-billed Chough	*Pyrrhocorax pyrhocorrax*
Ruppells Black Chat	*Myrmecocichla (Pentolaea) melanea*
Spot-breasted Plover	*Vanellus melanocephalus*
Thick-billed Raven	*Dendropicos abyssinicus*
Wattled Ibis	*Bostrychia carunculata*
White-backed Black Tit	*Parus leuconotus*
White-billed Starling	*Onychognathus albirostris*
White-collared Pigeon	*Columba albitorques*
White-winged Cliff Chat	*Myrmecocichla semirufa*

2.2.5. Socio-economic status of the people in and around SMNP

The local communities in and around SMNP practice mixed farming as major source of income and employment. Livestock farming and other off-farm economic activities supplement crop production, as crop production alone can not sustain their livelihood.

Simien Mountains National Park shares administrative boundaries with five administrative districts (woredas); namely: Debark, Janamora, Adi Arkay, Beyeda and Tselemet. The North Gondar Administrative Zone where SMNP is located has the largest livestock resource. In the Simien Mountains, people rely more on livestock for securing the household livelihood because crop production is low and because of the high altitude and poor soil qualities (AWF 2015).

Currently, out of the total 41,200 ha of the Park area, 12,047.18 ha (29.29 %) is overgrazed mainly by livestock coming from the neighboring woredas (districts) of Debark, Janamora and Beyeda. More than 40% of SMNP is highly disturbed by anthropogenic activities, 62.9% of the grassland area is intensively grazed by livestock and 8.7% is currently under cultivation largely for barley and wheat. Households from all villages around the park use the park for grazing livestock. The Gich plateau, which is located in the central part of SMNP is heavily overgrazed. These land- uses have contributed significantly to a reduction and degradation of key habitats and likely impacted key ecological processes of the Simien Mountains (AWF 2015).

2.3. Guassa Community Conservation Area

Guassa Community Conservation Area is located in North Shoa Zone, Gera- Keya Woreda of the Amhara Natonal Regional State, Ethiopia. It is situated in the central highlands on the rift valley escarpment between latitudes 10 º 15'–10 º 27' N and longitudes 39 º 45'–39 º 49' E with elevation ranging from 3,200 to 3700 m a.s.l. It is 265 km northeast of Addis Ababa (Fig 8) (Habtamu wodaj *et al.* 2016). Guassa Community Conservation Area covers an area of 111 km^2 (Zelalem Tefera *et al.* 2004, 2005). It was formerly under a communal management system known as 'Qero', which was established by the pioneer Fathers called "*Aqgni Abat*" in Menz Asbo and Gera in the 17th century and lasted for more than 400 years, for the protection of the grasslands in the Guassa area of Menz in the central highlands of Ethiopia.This system was reduced as a result of the agrarian reform of 1975 under the Derg regime (GEF 2007). The system sustainably protected the ecosystem and the system is now reinitiated as Guassa Community Conservation Area (GCCA) in 2008 (Zelalem Tefera and Leafder-Williams 2006).

2.3.1. Geology and Soil

The formation of Guasa area during the Oligo-Miocene was a result of tectonic and volcanic activities (Zanettin and Justen-Visentin 1974; Zelalem Tefera 2000). Lava covered all the previous rock formations that had been formed prior to the formation of the Rift Valley. Guassa area contains 15–26 million year old Miocene rhyolites and basalts, sometimes referred as an Alaji-Molale formation and 20–26 million year old Oligo-Miocene basalts and Phonolites (Zanettin and Justen-Visentin 1974; as cited in Zelalem Tefera 2000).

The central highland soil is characterized by two principal types, originating from the disintegration of volcanic substrates intermingled with sand and limestone. These comprise: black clay soil (vertisols) and reddish brown heavy loam (redsoil). The former type appears on flat plateau along the bottom of valleys. The latter appears on valley slopes and well-drained areas. Generally, the soil in Guassa area is deep. However, on higher ground, the soil is shallow and highly mineralized (Zanettin and Justen-Visentin 1974).

2.3.2. Climate

The climate of Guassa varies with altitudinal gradient and seasonal changes. Rainfall is bimodal with long wet season from the end of June to September and a short wet season in March and April. Mean annual rainfall and temperature is 917 mm and 12.8^{o}C, respectively. The temperature varies between –7^{o}C to 26^{o}C (EWNHS, 1996; Zelalem Tefera *et al.* 2012).

2.3.3. Vegetation

The GCCA is characterized by hills and valley bottoms interspersed with swamps and open areas of montane and African redwood (*Hagenia abyssinica*), *Olea europaea* sub sp. *cuspidata*, African pencil cedar (*Juniperus procera*), Outeniqua yellowwood (*Podocarpus falcatus*), *Allophyllus abyssinicus*, fescue (*Festuca* spp.), *Erica arborea*, Curry bush (*Hypericum revolutum*), Giant lobela spp., thyme *Thymus* spp. are the main plant species found in GCCA (Zelalem Tefera *et al.* 2005).

2.3.4. Fauna

Ethiopian wolf (*Canis simensis*), Gelada baboon (*Theropithecus gelada*), Abyssinian hare (*Lepus starcki*), Unstriped grass rat (*Arvicanthis abyssinicus*) and Abyssinian meadow rat (*Stenocephalemus grisecauda*) are the endemic mammals at GCCA. Table 4 lists these and other species of mammals in GCCA (Yalden and Largen, 1992; in Zelalem Tefera, 2000; EWCA 2013).

Guassa Community Conservation Area is known to harbor 26 highland biome bird species. Of these, Spot-breasted Lapwing (*Vanellus melanocephalus*), Rouget's Rail (*Rougetius rougetiI*) Abyssinian Longclaw (*Macronyx flavicollis*) and Black-headed Siskin *(Serinus nigriceps*) are Ethiopian endemic birds (EWNHS 1996).

Table 4. Mammal species found in GCCA

Common name	**Species name**
Grey duiker	*Sylvicapra grimmia*
Klipspringer	*Oreotragus oreotragus*
Spotted hyaena	*Crocuta crocuta*
African civet	*Civeta civettictis*
Ratel/ Honey badger	*Melivora capensis*
Egyptian mongoose	*Herpestes ichneumon*
Serval cat	*Felis serval*
Ethiopian wolf	*Canis simensis*
Gelada baboon	*Theropithecus gelada*
Abyssinian hare	*Lepus starcki*
Porcupine	*Hystrix cristata*
Common mole rat	*Tachyoryctes splendens*
Unstriped grass rat	*Arvicanthis abyssinicus*
Abyssinian meadow rat	*Stenocephalemus grisecauda*
Groove-toothed rat	*Otomys typus*

2.3.5. Socio-economic status of the people around GCCA

Mixed farming (crop production and livestock husbandary) is the dominant economic activity of the people in the area. Barley, beans and lentils are commonly grown in the area. Barely is the main subsistence crop. Large herds of sheep are reared and used for meat and wool production. The main farming animals are oxen, although horses and donkeys are used sometimes (Zelalem Tefera 2000).

Guassa Community Conservation Area provides resources such as fodder, fuelwood, building materials, household farming materials, water and fertile soil. *Erica arborea, Hypericum revolutum, Thymus shimperi and Festuca* spp. are common in the area. *Erica arborea* and *Hypericum revolutum* are collected for fuelwood. *Thymus shimperi* is used to flavor drinks and food. The *Festuca* spp. is periodically harvested and sold by the local people for thatching purpose. Guassa Community Conservation Area also serves as refuge for the livestock around the area during severe drought (NFEP 2004).

2.4. Study period

A preliminary survey was conducted in January to February, 2014 to identify flocking sites of the Ankober Serin in both study areas. A total of four field data collection sessions were employed in both SMNP and GCCA. Wet season data collection was conducted between July 1 – August 15, 2014 (1st wet season) and July 1 – August 15, 2015 (2nd wet season). The dry season data were collected between January 1–February 15, 2015 (1st dry season) and January 1–February 15, 2016 (2nd dry season). Each data collection session lasted around 15 days per study area.

2.5. Methods

2.5.1. Selection of flocking sites

SMNP

Extensive search of birding areas at SMNP was carried out during the priliminary study period starting from the entrance of Debark to Lemalimo and from Sankaber to Sebat Minch (Mesareria). The latter trip was conducted by slow walking along the highway in serach of Ankober Serin flocking sites. Finally, flocks were detected at Chenek, Bwahit and Mesareria (Table 5). The flocking sites are located on map in figure 7.

Table 5. Location, elevation and local names of the five flocking sites selected at SMNP.

No	Flocking site	GPS Location	Elevation	Local area names	Area (km^2)
1	Simien flocking site I (SFS I)	13º 15' 13.5" N–13 º 15'11.4"N, 038º10' 59.2"E– 038 º 10'58.8"E	3533–3502	Chenek	0.5
2	Simien flocking site II (SFS Il)	13°15'26.50"N–13°15'29.50"N, 38°11'15.59"E–38°11'14.10"E	3502–3509	Chenek	0.4
3	Simien flocking site III (SFS III)	13°15'32.49"N–13°15'43.30"N, 38°13'55.10"E–38°13'44.83"E	3972–3794	Bwahit	0.3
4	Simien flocking site IV (SFS IV)	13 º 12' 54.4"N–13 º 12'58.9"N, 38 º 13' 17.7"E– 038 º 13' 24.1"E	4233–4297	Mesareria	0.7
5	Simien flocking site V (SFS V)	13 º 11'57.9" N –13 º 13'23.3" N 38 º 13' 01.4" –E 038 º 13' 23.3" E	4201–4292	Mesareria close to Sebat Minch	0.75

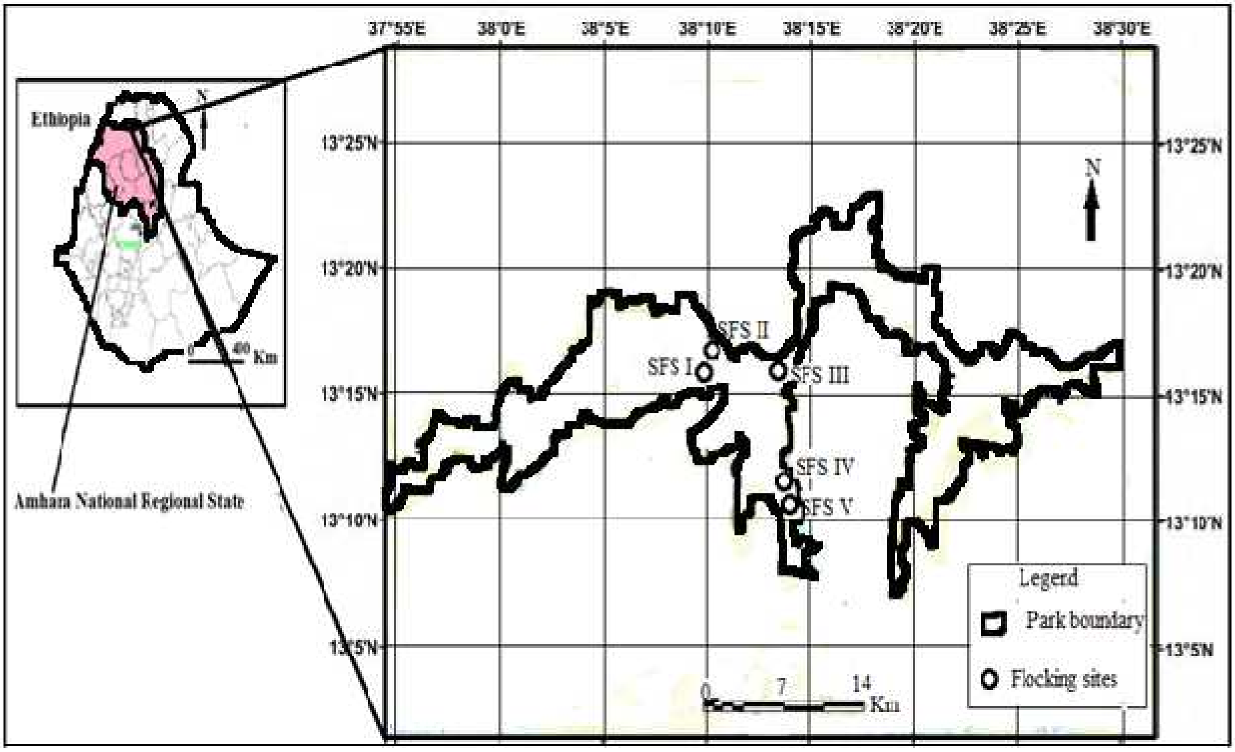


Figure 7. Location of the five flocking sites at SMNP (SFS I to SFS V).

GCCA

At GCCA, the road from the conservation area to Mehal Meda town (about 20 kms) was searched to find flocking sites of Ankober Serin. Another road from Mehal Meda to a village school called Addis Alem (about 15 kms) was also searched in a similar manner. Finally, Ankober Serin flocks were identified at the cliffs near villages called Yegora, Difrege, Metki, Kebelle 20, and Addis Alem (Table 6). The identified flocking sites in GCCA are located on the Map shown in figure 8.

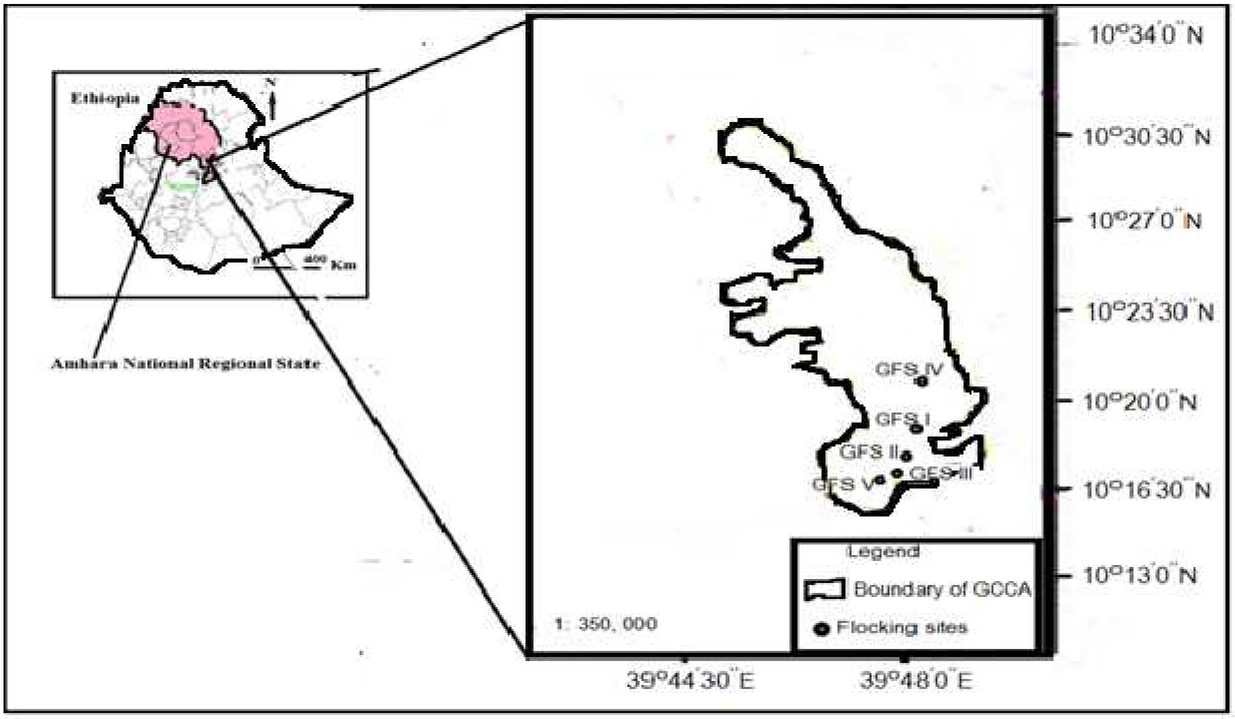


Table 6. Location, elevation and local names of the five flocking sites selected at GCCA

No	**Flocking site**	**GPS Location**	**Elevation (m)**	**Local area names**	**Area (km^2)**
1	Guassa flocking site I (GFS I)	10°18'15.8"N –10°18'18.0"N, 39°48' 37.7"E–39°48' 37.8"E	3156–3252	Yegora	0.35
2	Guassa flocking site II (GFS II)	10° 17' 07.3" N–10°17'06.3" N, 39°48" 37.9"E–39° 48' 33.4" E	3214–3155	Difrege	0.25
3	Guassa flocking site III (GFS III)	10° 16' 50.3" N–10° 17' 41.1" N, 39°47' 57.4" E–39°47' 59.8" E	3284–3345	Metki	0.30
4	Guassa flocking site IV (GFS IV)	10° 20'5.25" N–10° 20'5. 23" N, 39° 48' 20.00" E–39° 48'24.61"E	3388–3439	Addis Alem	0.25
5	Guassa flocking site V (GFS V)	10° 16' 16.08" N–10° 16' 13.33" N, 39° 47' 19.70" E – 39° 47'9.94" E	3275–3258	Kebele 20	0.25

Figure 8. Location of the flocking sites at GCCA (GFS I to GFS V)

2.5.2. Flock size

As Ankober Serin is a gregarious species that occurs in flocks of up to 50 or more individuals (BirdLife International 2012), its population can be better estimated using the flock count method. Thus, the flock size was determined using the flock count method (Sutherland 2004). Flocks were counted at the pre-identified flocking sites. The flocking sites were a minimum of 1 km apart from each other. Counting was carried out in the morning (06:00–08:00h). The team was composed of four individuals (The researcher, a field expert and two field assistants from each study areas). Two individuals formed a counting team. The team counted the birds independently and the average count was recorded to minimize human error. Each flocking site was sampled for three consecutive days during each sampling session. There were four sampling sessions during the study: two wet season and two dry season counts for both SMNP and GCCA.

Occasionally, *S. ankoberensis* flocks were found mixed with other species. In those cases, it was necessary to get closer enough to about 20 m to ensure counting only Ankober Serin without disturbing the flock. In some cases, the congeneric species, Brown-rumped Seed eater (*S. tristriatus*) and Streaky Seed eater (*S. striolatus*) (Fig. 9A and B) were found to confuse observers at a distance. But, Ankober Serin was easily distinguishable by its heavy streaks and absence of the supercilium with closer observation.

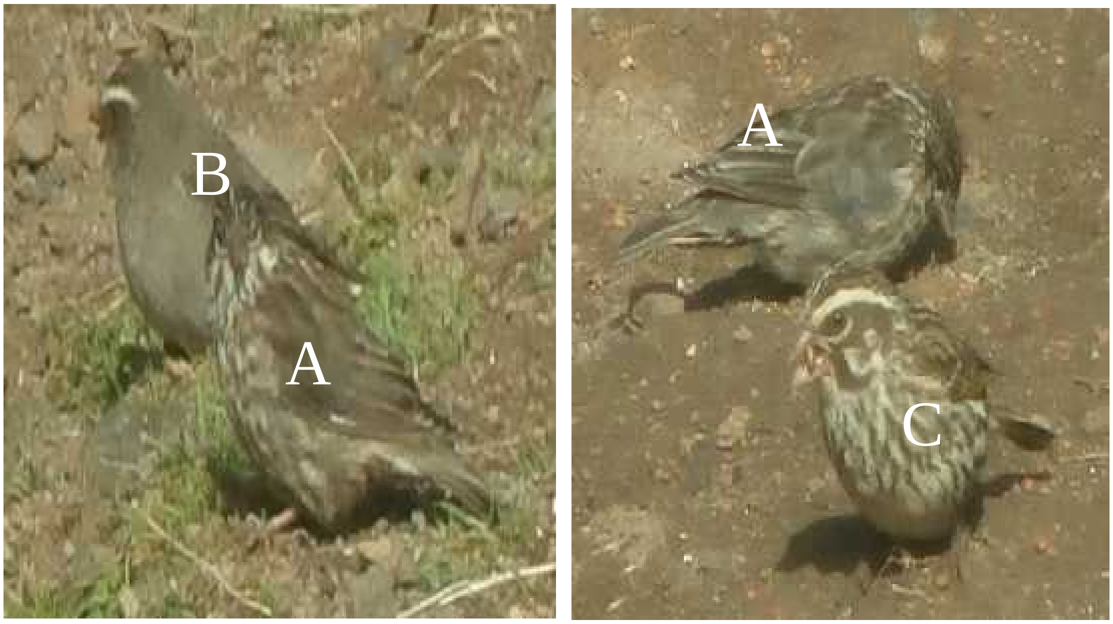


Figure 9. (A) Ankober Serin mixed with (B) Brown-rumped Seed eater (*Serinus tristriatus*) and (C) Streaky Seed eater (*Serinus striolatus*) (Photo: Abebayehu Desalegn 2015)

2.5.3. Habitat disturbance and level of encroachment

The extent of disturbance was evaluated by designating score of disturbance levels: Low disturbance; if habitat is with no human or cattle encroachment, Medium disturbance; if only foraging ground is disturbed whilst roosting cliffs are beyond the reach of human and livestock, and High disturbance; if both roosting cliffs and foraging grounds are disturbed by livestock and humans) (Modified from Yang *et al* 2015).

2.5.4. Habitat analysis

Vegetation analysis of Ankober Serin habitat was conducted using the method of Wiens and Rotenberry (1981). One third of the flocking sites i.e two sites out of five from each study area were selected based on their accessibility (GFS I and GFS II from GCCA; SFS I and SFS IV from SMNP). Vegetation sampling was conducted only for the wet season since most of the herbs dry out during the dry season. At SFS IV, 100 m transect line passing through the center of the flocking site was laid and perpendicular lines of 30 m length on either side of the transect at 10 m intervals were marked. Sampling points were established on each perpendicular line at 10 m intervals where the foraging ground is larger at SFS IV (Fig. 10). In the reminaing three flocking sites, though the same design was followed, the number of sampling points were <60 depending on the sizes of the foraging ground (Table 7).

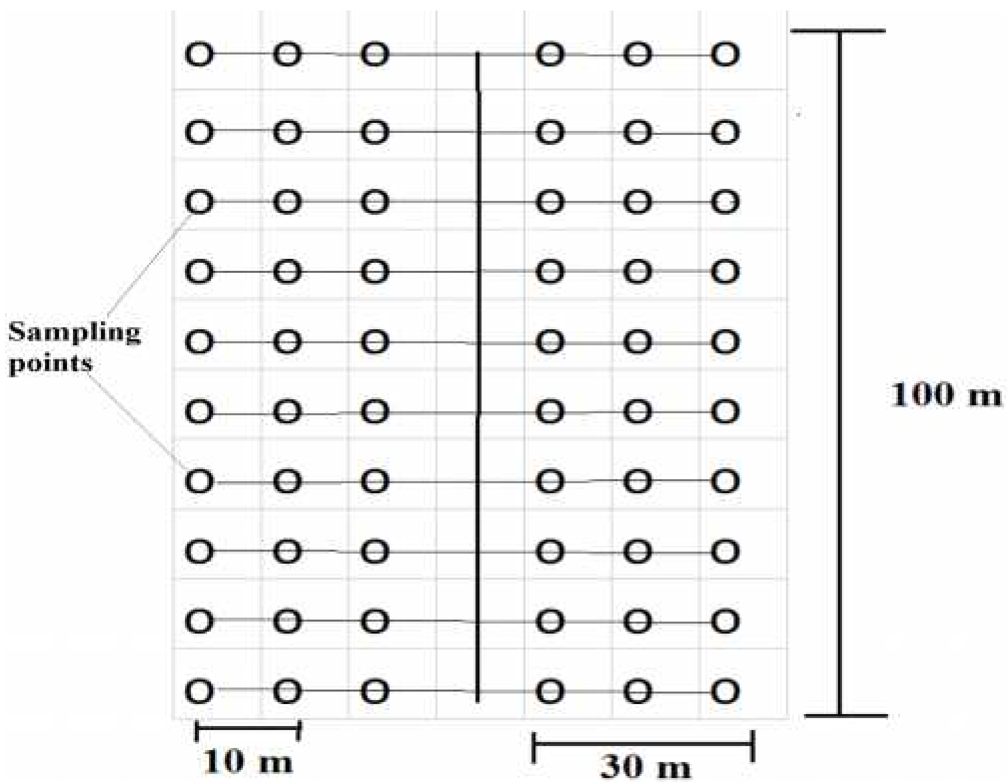


Figure 10. Sampling design for vegetation analysis

Table 7. Numbers and area of the sampling points

Flocking sites	Location	Number of sampling points	Area/m^2 of the foraging ground
GFS I	10°18'15.8"N –10°18'18.0"N, 39°48' 37.7"E–39°48' 37.8"E	18	0.0018 km^2
GFS II	10° 17' 07.3" N–10°17'06.3"N, 39°48" 37.9"E–39° 48' 33.4" E	12	0.0012 km^2
SFS I	13° 15' 13.5" N–13° 15'11.4"N, 38° 10' 59.2"E– 38° 10'58.8"E	30	0.003 km^2
SFS IV	13° 12' 54.4"N–13° 12'58.9"N, 38° 13' 17.7"E– 38° 13' 24.1"E	60	0.006 km^2

The following vegetation parameters were recorded from each of the sampling points.

I. ***Vegetation composition*** **–** plant species which occured within 2m radius of the sampling points were recorded. Those species commonly known were recorded directly with the help of experts, and some were photographed and identified at the National Herbarium, Addis Ababa University.

II. ***Physognomy and vegetation cover*** **–** the physiognomic characteristics of the vegetation and the cover (herb, shrub or bare ground) was analysed by calculating the percentage proportion of each physiognomic category at all sampling points. The vegetation cover was visually estimated at each sampling point (Sutherland 2004).

III. ***Vegetation diversity*** – diversity was calculated for each flocking site using the Shannon–Weiner diversity index:

$$N = -\sum pi \ln pi$$

Where; N= plant diversity

Pi= Abundance proportion of the i^{th} species (Shannon, 1949)

IV. ***Vertical stratification*** – the vertical stratification was measured at each sampling point by measuring distinct vegetation vertical strata with a measuring tape. The plant height strata were categorized as <5 cm, 5–10 cm, 11–20 cms, 21–30 cms, 31–40 cms, 41–50 cms, 51– 60 cms, 61– 70 cms, 71– 80 cms, 81–90 cms, 91– 100 cms, 101– 150 cms and 151– 200 cms and >200 cms (Weins 1973). The data were further re-categorized as <30 cms, 30–60 cms and above 60 cms for statistical analysis.

Additional information on vegetation characterstics of the cliffs and nest of Ankober Serin was recorded from visual observations at both study areas.

2.5.5. Diet composition

Undigested remains found in faecal droppings of Ankober Serin were used to determine its diet composition. Fresh Samples were collected from the four accessible flocking sites used in the vegetation analysis; GFS I, GFS II, SFS I and SFS IV. A minimum of 10 fresh faecal droppings were collected from each of the flocking sites of both study areas during dry and wet seasons. The diet composition was studied following the quantitative method of Robinson (2004). Pellets were stored in 70 % ethyl alcohol until they were analysed. For analysis, each pellet was dissolved in 3 ml of water and the contents were determined using a point-quadrat method through a 20X binocular microscope. For each sample, 0.1 ml of the suspension was pippetted into a shallow-welled microscope slide. This was then placed over a grid of 72 points (which filled the field of view) and the identity of food items falling on each grid point was determined. This procedure was then repeated five times for randomly selected, non-overlapping areas of the slide, giving a total of 360 grid positions counted for each sample. Faecal particles were recorded as seeds, leaves, insects and mosses. The proportion of a given faecal particle was calculated for each flocking site. Variations between flocking sites and study areas were assessed. Seasonal variation in diet composition was determined by comparing dry and wet season data.

2.5.6. Genetic Diversity

2.5.6.1. Blood sampling

Blood samples were collected from birds captured using mist nets (Fig. 11). Blood was taken by puncturing brachial vein with 28 gauge needles and preserved using FTA cards until DNA extraction was done at the University of Bonn, Germany. A total of 30 birds were netted (14 from three flocking sites in GCCA and 16 from other three flocking sites in SMNP). Permission on the use of live animal resources for this study was obtained from the Ethiopian Wildlife Conservation Authority (EWCA).

Figure 11. Ankober Serins captured using mist net (Photo: Abebayehu Desalegn 2015).

2.5.6.2. DNA Isolation

For isolation of DNA from FTA cards, QIAGENS DNA “blood and tissue micro kit” was used following the instructions for “dried blood spots” (version 12/2014). From the FTA cards, 3 mm diameter punches were cut and placed in a tube with 180µl buffer ATL and 20µl Proteinase K, and incubated for 1h at 56°C using a thermomixer. Then 200µl of buffer AL was added (including carrier RNA as recommended in the protocol). After two centrifugation/washing cycles, final elution was done with 30ml of buffer AE. Quality and quantity of DNA were estimated using a “nanodrop” UV-photometer.

2.5.6.3. PCR primers and conditions

For PCR amplification of microsatellite marker, published primer sequences were used (Melo and Hansson 2006). Initially, the primer pairs Ase42, Ase43, Ase48, Cuµ04, Cuµ28 and Gf08 were used. Eppendorf Thermocyclers were used for all PCRs. PCR conditions varied with the primers used, especially in annealing temperatures. These were adjusted after a series of test-PCRs. Primer specific annealing temperatures were: Ase42=60°C; Ase4=53°C; Ase48=55°C; Cuµ04=55°C; Cuµ28=58°C and Gf08=60°C. For using primers in fragment analysis, forward-primers were ordered with fluorescent dyes Cy5 and Cy5.5 attached to their 5'-ends. See Appendix 3 for Primer sequence information.

Basic PCR conditions were the following: initial denaturation (94°C for 2 min), followed by 40 cycles of denaturation (94°C, 30 sec), primer annealing (1 min, temperature primer dependent, primer elongation (1 min, 72°C). Finally, 1 min at 72°C finished the PCR, and the samples were cooled down to 4°C. All PCR kits used were from 5prime (formerly Eppendorf). PCR setup was optimized for 25µl samples. All PCRs were set up on a single PCR product experiments (no multiplex PCR). Mastermix included Hot MasterTaq (0.25µl per 25µl PCR), 10 nm dNTPS and buffer with magnesium. PCR success was tested by visual inspection after separation on a 1% agarose gel.

2.5.6.4. Fragment analysis

Fragment size analysis was done using a Beckman Coulter CEQ8000 genetic analyzer. Polymerisation chain reaction products were diluted at 1:20 in 40µl of sample loading solution, adding 0.5µl of size standard "400" (Beckman Coulter). Fragment separation was done on CEQ8000 with separation programme "frag-B". Initial visualization and estimation of fragment sizes was done using the GenomeLab software delivered with the CEQ 8000 system. One of the estimated fragment sizes is shown in figure 12.

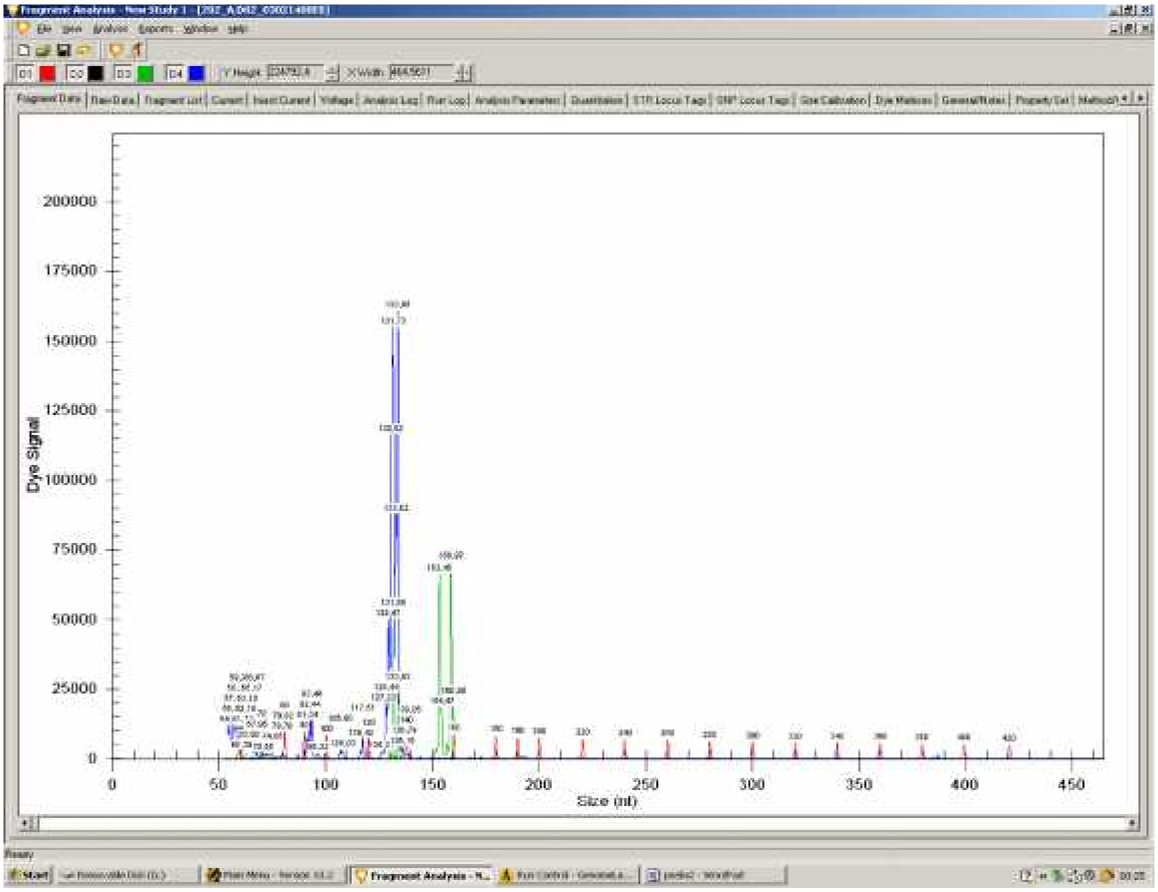


Figure 12. A screenshot from CEQ8000, red are the size markers, green and blue are two different PCR products, both are heterozygous (two major peaks each).

2.6. Statistical Analyses

Variations in flock size, ground cover, and vegetation structure, vegetation height, dietary composition within and between the study areas were compared using ANOVA. Pearson correlation coefficient was used for correlations of flock size and the habitat parameters as disturbance, ground cover, vegetation structure and vegetation height. Variation of vegetation diversity of flocking sites was analysed using Shannon Weiner diversity index and compared using diversity t-test.

The genetic analysis included the genic and genotypic differentiation (according to exact G tests). Genic differentiation tests were used for differences between two populations by means of different sets of allels present. Genotypic differentiation ("exact G test") was a similar test like the genic test but checking different genotypes (allele combinations from the two chromosomes).

The Hardy-Weinberg equilibrium was calculated and the deviation from the expected equilibrium was assessed using the exact test.

3. Results

3.1. Flock size

The mean number of Ankober Serin for five flocking sites for the 1st and 2nd wet seasons was 196 ±11.31 (GCCA) and 238±4.24 (SMNP); and it was 285.5±31.82 (GCCA) and 421±17.68 (SMNP) for the 1st and 2nd dry seasons (Table 8).

Table 8. Ankober Serin flock size during wet and dry seasons in GCCA and SMNP (2014–2016)

FLOCKING SITES	1st Wet	2nd Wet	Mean	1st Dry	2nd Dry	Mean
GFS I	55	46	50.5	76	90	83
GFS II	36	34	35	25	34	29.5
GFS III	46	40	43	62	60	61
GFS IV	52	50	51	70	84	77
GFS V	15	18	16.5	30	40	35
SFS I	60	50	55	70	78	74
SFS II	35	25	30	50	40	45
SFS III	12	20	16	54	36	45
SFS IV	70	60	65	120	135	127.5
SFS V	64	80	72	140	120	130

The mean flock size of Ankober Serin for the five flocking sites in GCCA for both seasons was 48.15±20.8 and that of SMNP was 65.95±37.3. The difference in flock size between the study areas was insignificant ($F_{1, 38}$= 3.472 p=0.070) (Fig. 13).

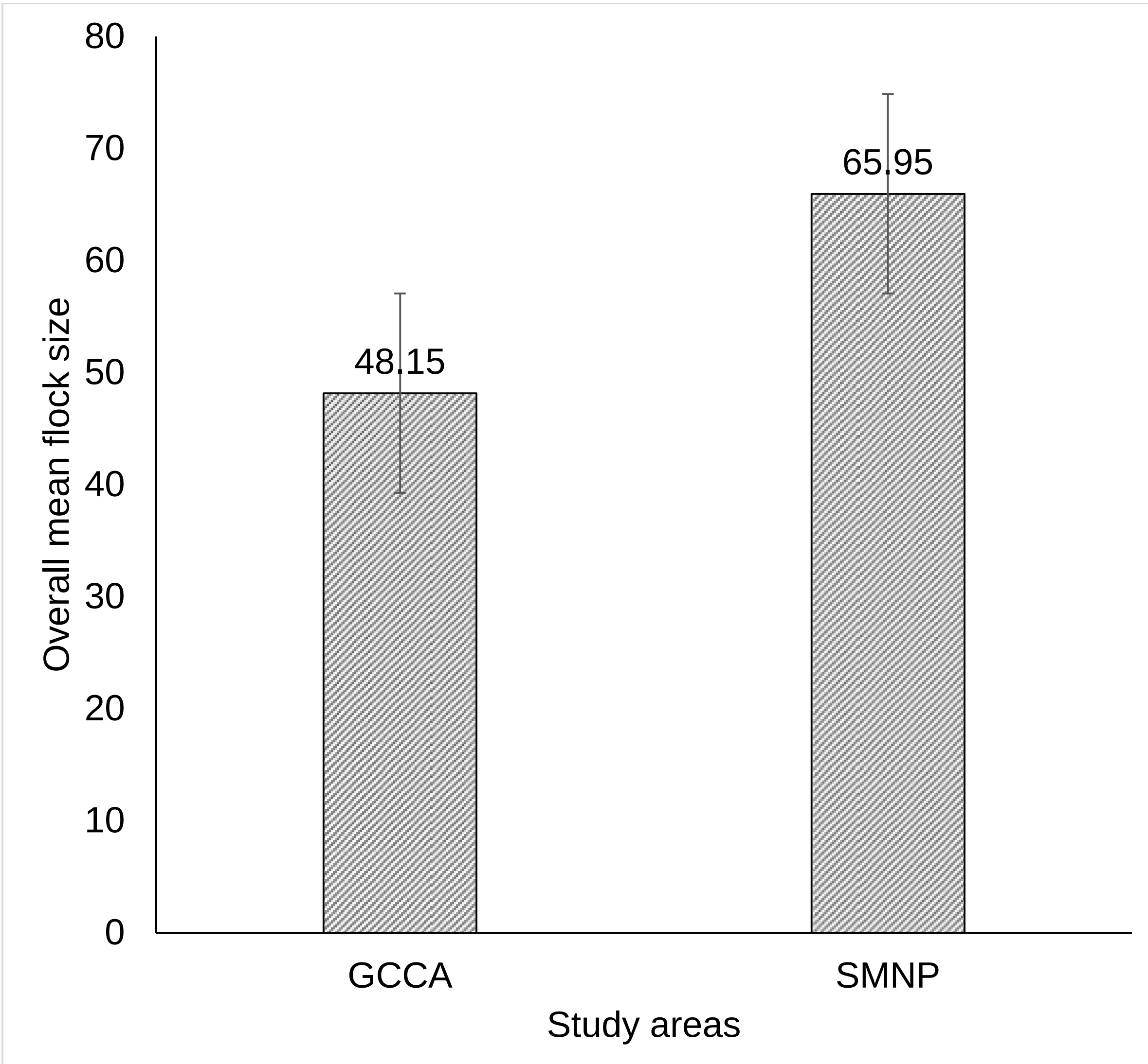


Figure 13. Mean flock size at GCCA and SMNP

3.1.1. Comparison of flock size between flocking sites

There was significant variation in flock size between all the flocking sites ($F_{9, 30} = 6.882$ $p=0.000$) with the highest average flock size recorded at SFS V (101±35.1) and the lowest at GFS V (25±75) (Fig. 14). Comparison of flock size between flocking sites within each study area revealed significant variation (SMNP, $F_{4, 15} = 7.362$ $p=0.002$) and (GCCA, $F_{4, 15} = 6.611$ $p=0.003$).

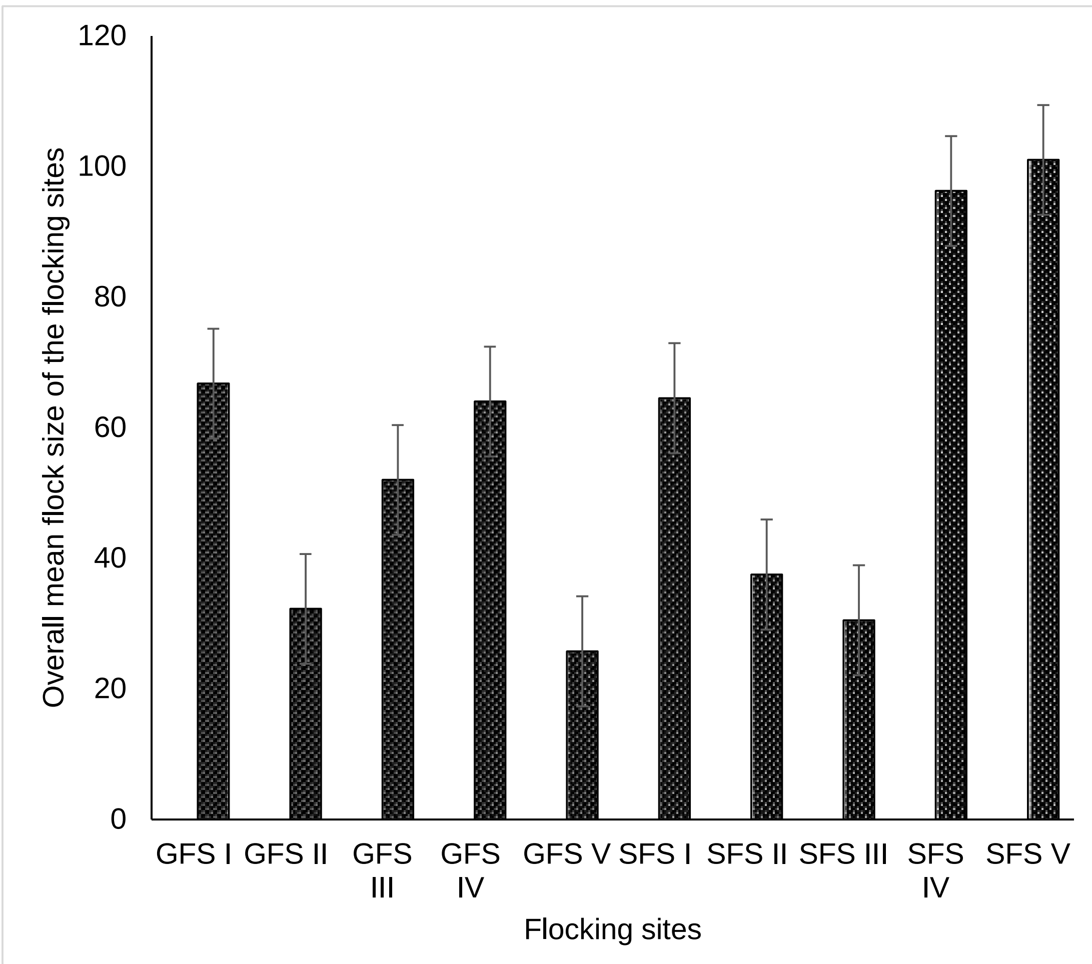


Figure 14. Mean ± SD flock size at all of the flocking sites

3.1.2. Seasonal variation in flock size

The overall mean flock size in the wet and dry season was 43.4 ±4.3 and 70.7± 7.9, respectively. The dry season flock size was significantly higher than the wet season ($F_{1, 38}$= 9.319 p=0.004) (Fig. 15).

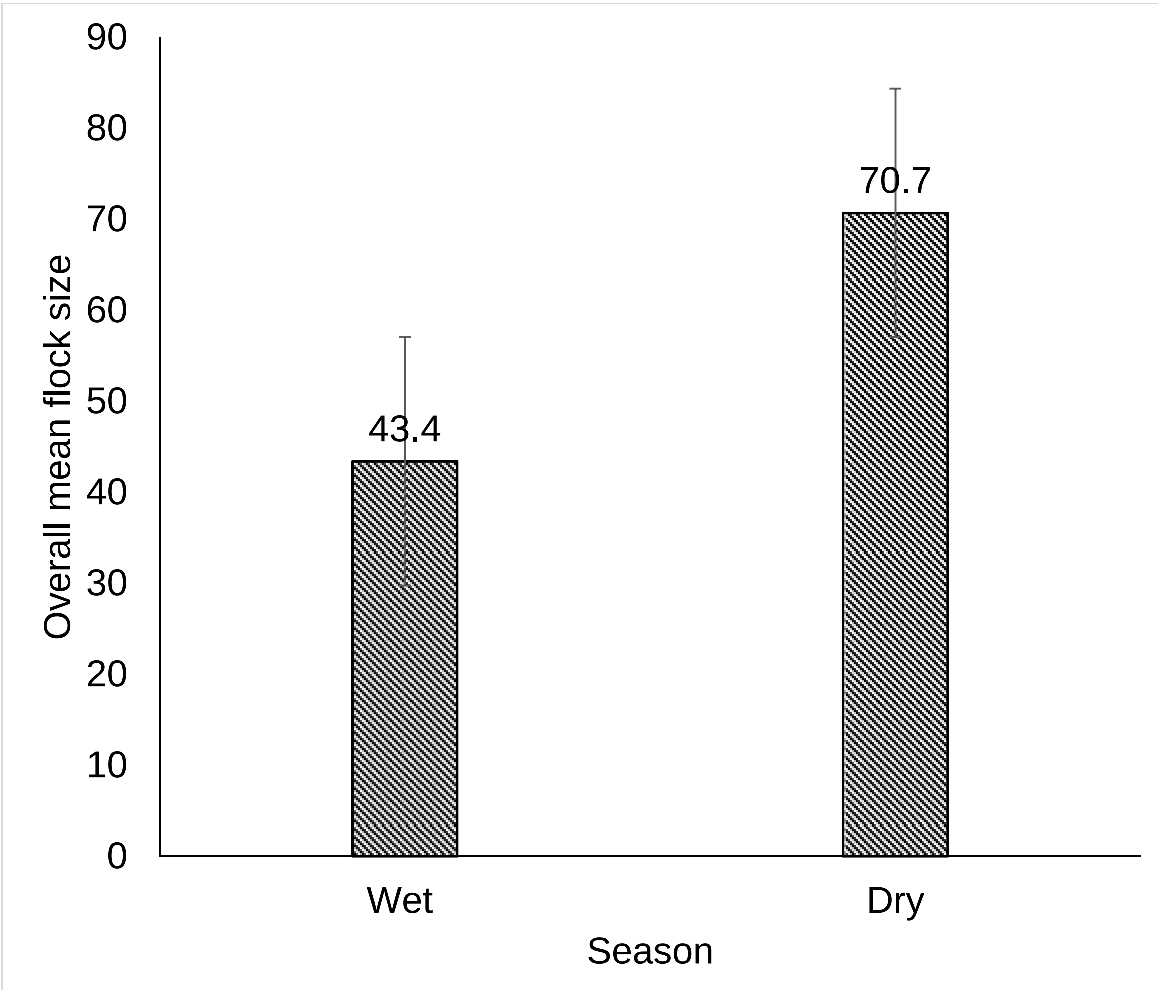


Figure 15. Mean ±SD flock size in the wet and dry season

3.2. Habitat disturbance

GFS II and GFS V had High-Level of disturbance; SFS IV and SFS V had Low-Level of disturbance while the rest had medium-level of disturbance (Table. 9).

Table 9. Level of habitat disturbances and overall mean flock size of the flocking sites

Flocking sites	Level of disturbance			Mean flock size±SD
	Low	Medium	High	
GFS I		✓		66.75±19.96
GFS II			✓	32.25±4.92
GFS III		✓		52.00±10.7
GFS IV		✓		64.00±16.1
GFS V			✓	25.75±11.5
SFS I		✓		64.50±12.15
SFS II		✓		37.50±10.41
SFS III		✓		30.50±18.57
SFS IV	✓			96.25±36.83
SFS V	✓			101.00±35.1

3.2.1. Correlation between habitat disturbance and flock size

Flock size was negetively correlated with level of disturbance ($r=-.876^{**}$ $p= 0.01$). Higher flock size was associated with low disturbance and lower flock size was associated with higher disturbance. Larger flock sizes were recorded at SFS IV and SFS V of SMNP whereas the smaller flock sizes were recorded at GFS II and GFS V of GCCA and SFS III of SMNP (Fig. 16).

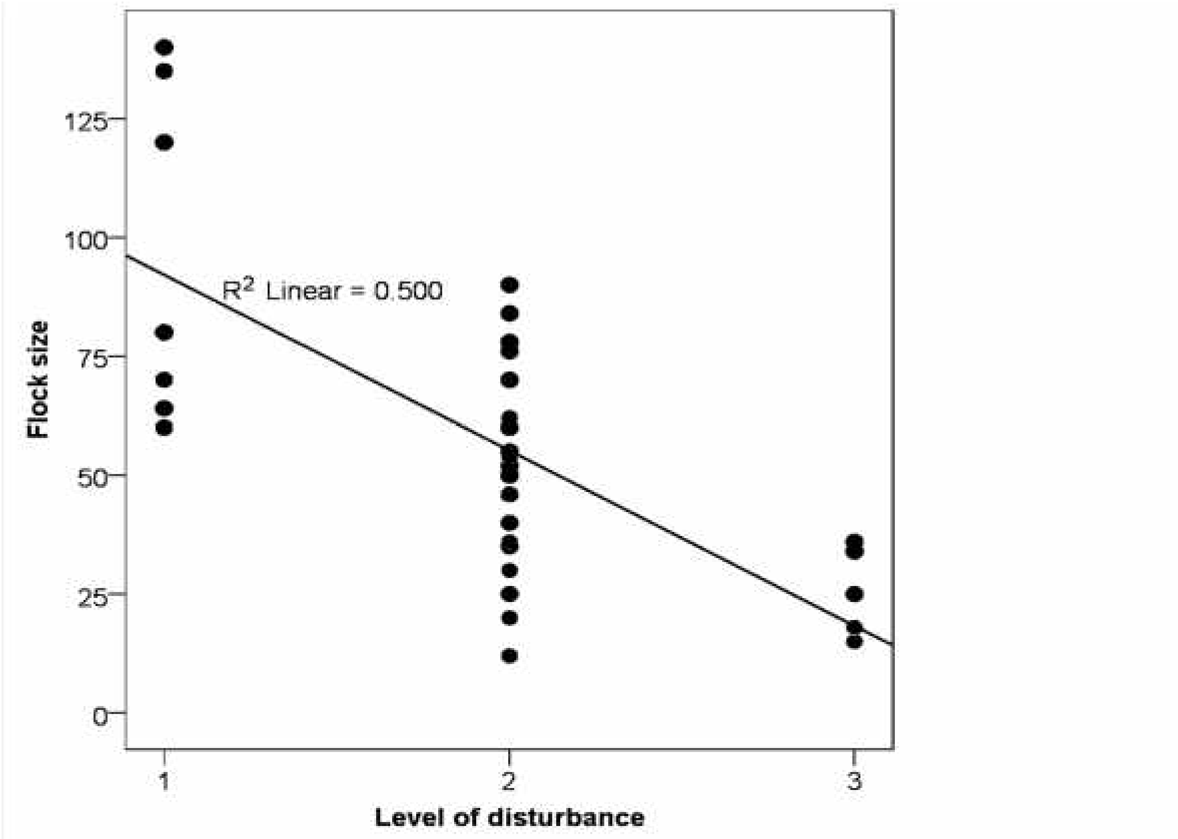


Figure 16. Correlation between level of habitat disturbance and flock size (1=low, 2=medium 3=high).

3.3. Vegetation characterstics

3.3.1. Vegetation composition

GFS I

Cynodon spp. and Andropogon spp. contributed more than 17% of the vegetation cover at this site while Alchemilla abyssinica, Crassula alba, Urtica simensis were <5%. The total number of plant species recorded in this site was 14 (Fig. 17).

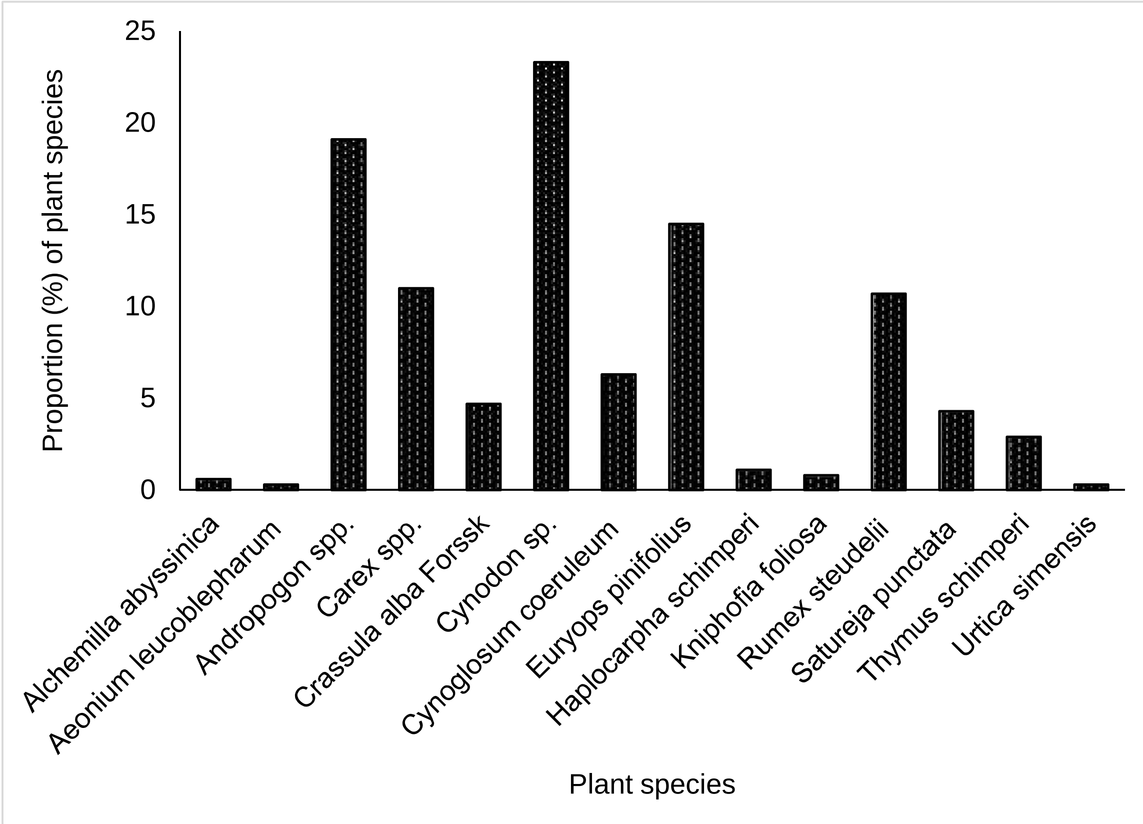


Figure 17. Percentage composition of plant species at GFS I

GFS II

Cynodon spp. and *Kniphofia* foliosa had proportions >17% while *Cardus shimperi, Europs pinifolius, Salvia merjamie and Urtica simensis* had proportions <5% at the foraging ground in GFS II. The total number of species in this site was similar to GFS I (n=14) (Fig. 18).

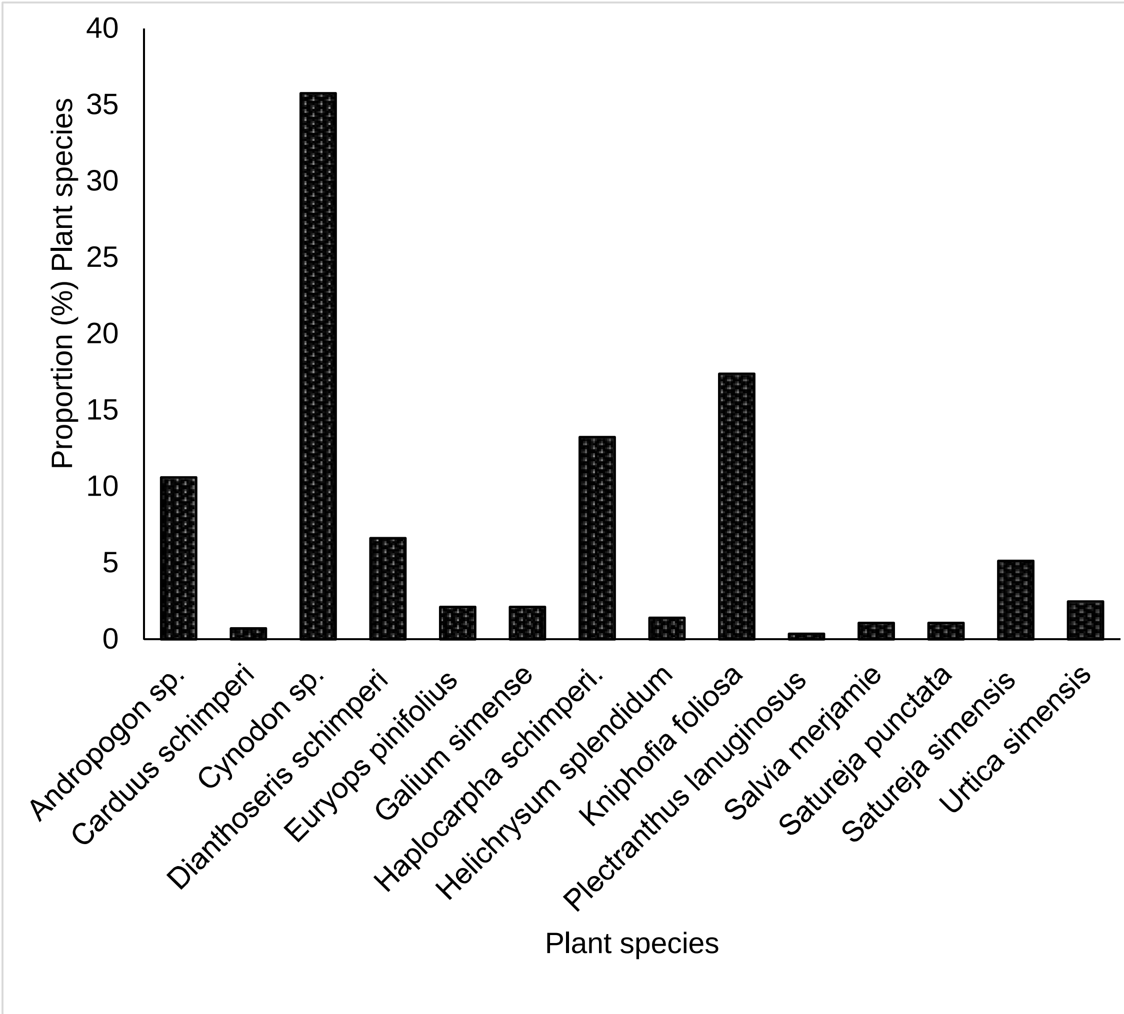


Figure 18. Percentage composition of plant species at GFS II

SFS I

Andropogon spp. and *Thymus schimperi* had proportions >29 % while *Kniphofia foliosa, Lobelia rhyncopetalum* had proportions <5% at the foraging ground in SFS I. The number of plant species in this foraging ground was 16 (Fig. 19)

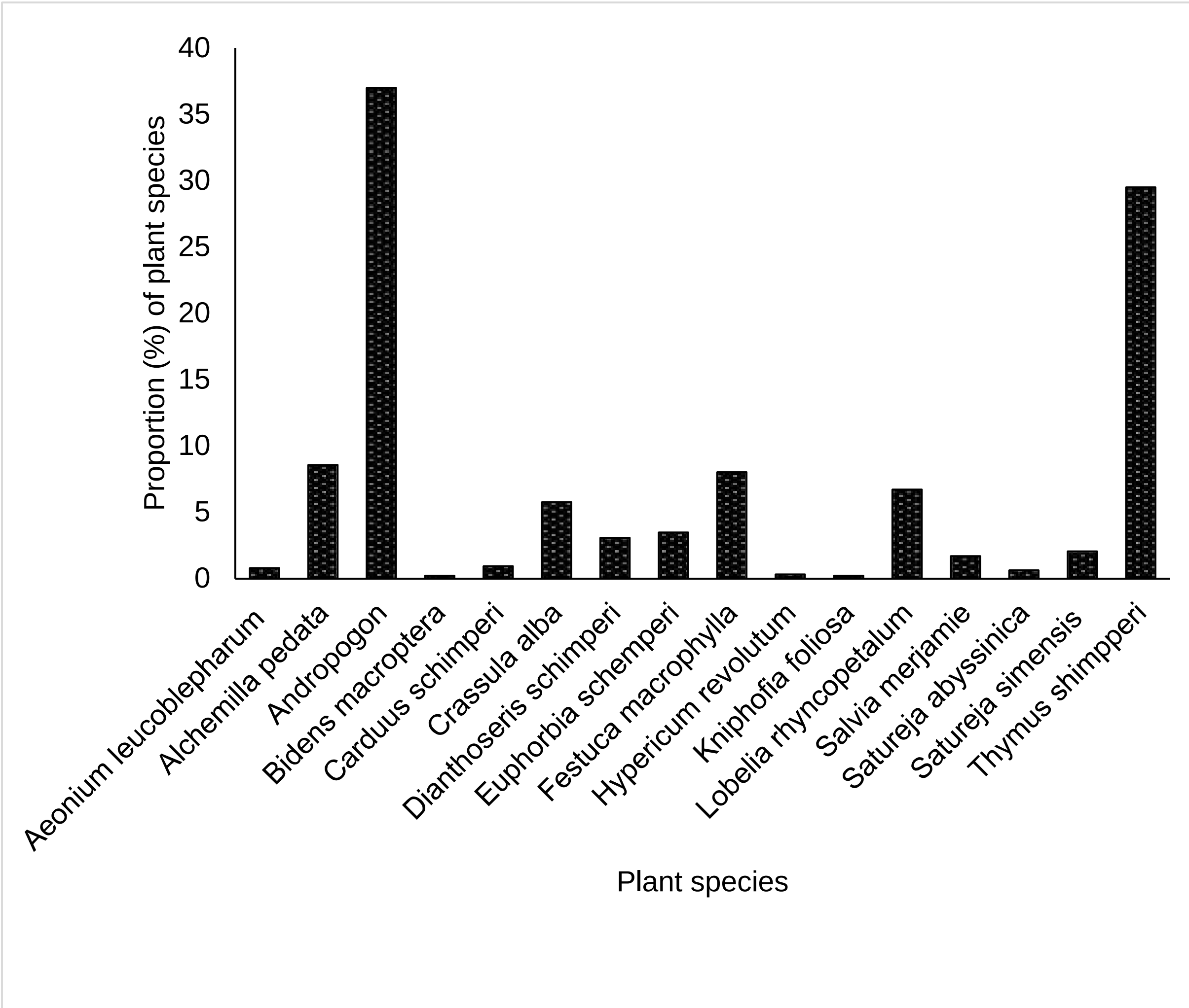


Figure 19.Percentage composition of plant species at SFS I.

SFS IV

Festuca macrophylla had proportion > 49% followed by *Thymus Shimperi* while *Agrostis quinquesta, Agrostis sclerophylla* and *Alchemilla bachitii* had proportions <5% at the foraging ground in SFS IV. The number of plant species in this foraging ground was 18 (Fig. 20).

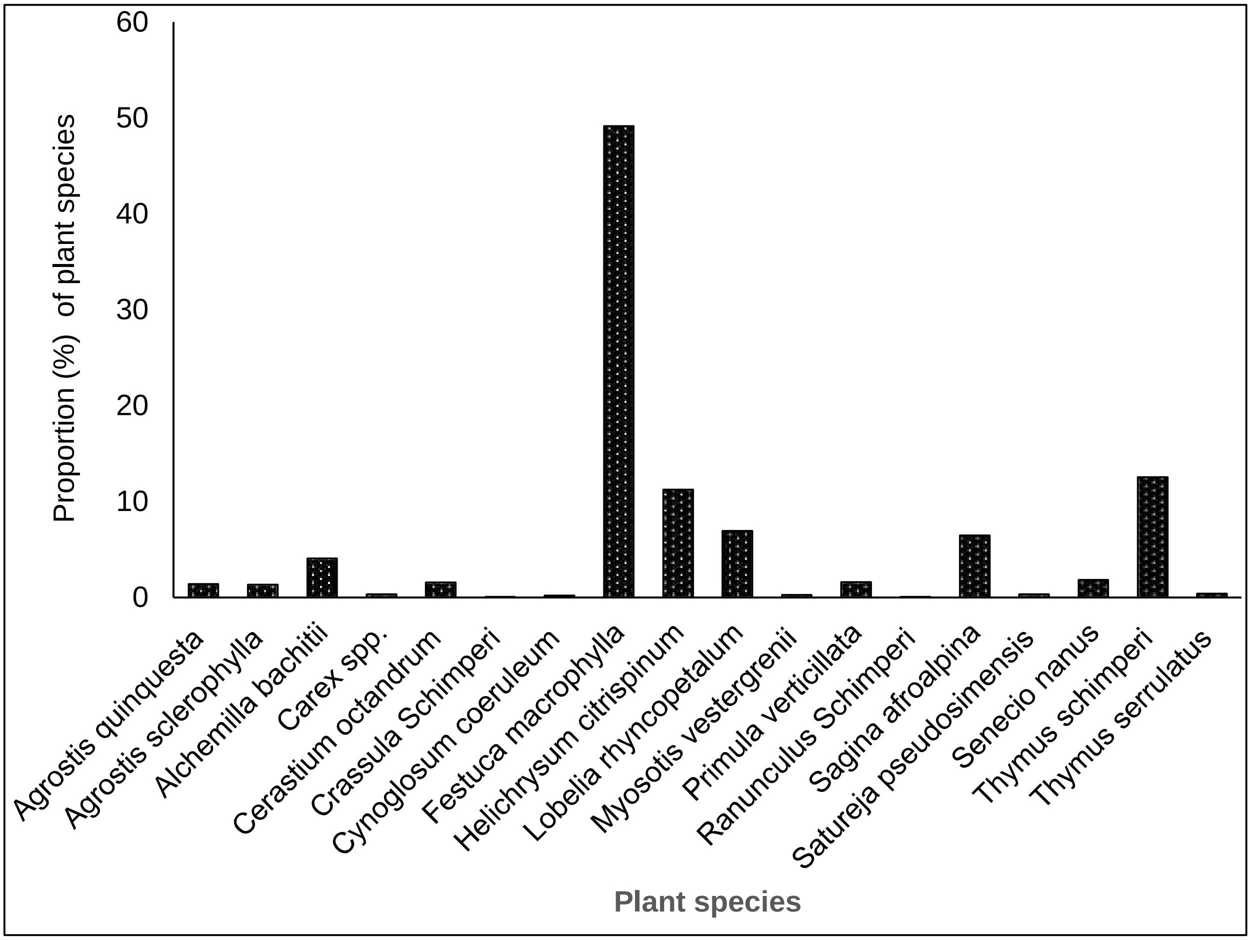


Figure 20. Percentage composition of plant species at SFS IV

3.3.1.1. Vegetation composition and flock size

Flock size showed significant correlation with the *Festuca* spp. and *Helichrisum citrispinum* dominated habitat (see SFS IV) and negative correlation with *Cynodon* spp. and *Haplocarpha shimperi* dominated habitat (see GFS II) (r=.457 p= 0.075) (Table 10).

Table 10. Dominant plant species and average flock sizes at the four flocking sites included in the vegetation analysis.

Flocking sites	Dominant plant species	Ovrall mean flock size
GFS I	*Cynodon* spp.	66.75±20
GFS II	*Cynodon* spp.	32.25±4.9
SFS I	*Andropogon* spp.	64.5± 12.2
SFS IV	*Festuca* spp.	96.25±36.5

3.3.2. Plant species diversity

The highest vegetation diversity values were obtained at GFS I, H=2 .115 (1st wet season) and H=2.129 (2nd wet season) while the lowest values were obtained at SFS IV, H=1.812 (1st wet season) and H=1.773(2nd wet season) (Table 11). The difference in the vegetation diversity between the two GCCA flocking sites (GFS I and GFS II) was statistically significant ($t_{(1241)} = 2.0229$, $p = 0.04$). Similarly, the mean flock size was higher at GFS I than GFS II. The difference in the vegetation diversity between the two SMNP flocking sites (SFS I and SFS IV) was not statistically significant ($t_{(4931)} = -0.2154$, $p = 0.8$).

Table 11. Plant diversity index of the foraging grounds of the four flocking sites

Flocking sites	Shannon–Weiner diversity index values		Ovrall mean flock size ± SD of the flocking sites
	Wet 1	Wet 2	
GFS I	2 .115	2.129	66.75±20
GFS II	1.869	1.849	32.25±4.9
SFS I	2.063	1.966	64.5± 12.2
SFS IV	1.812	1.733	96.25±36.5

3.3.3. Ground cover

In all the foraging grounds of the flocking sites, herbs were dominant (>50%) followed by bare ground (>20% in most flocking sites) (Fig 21).

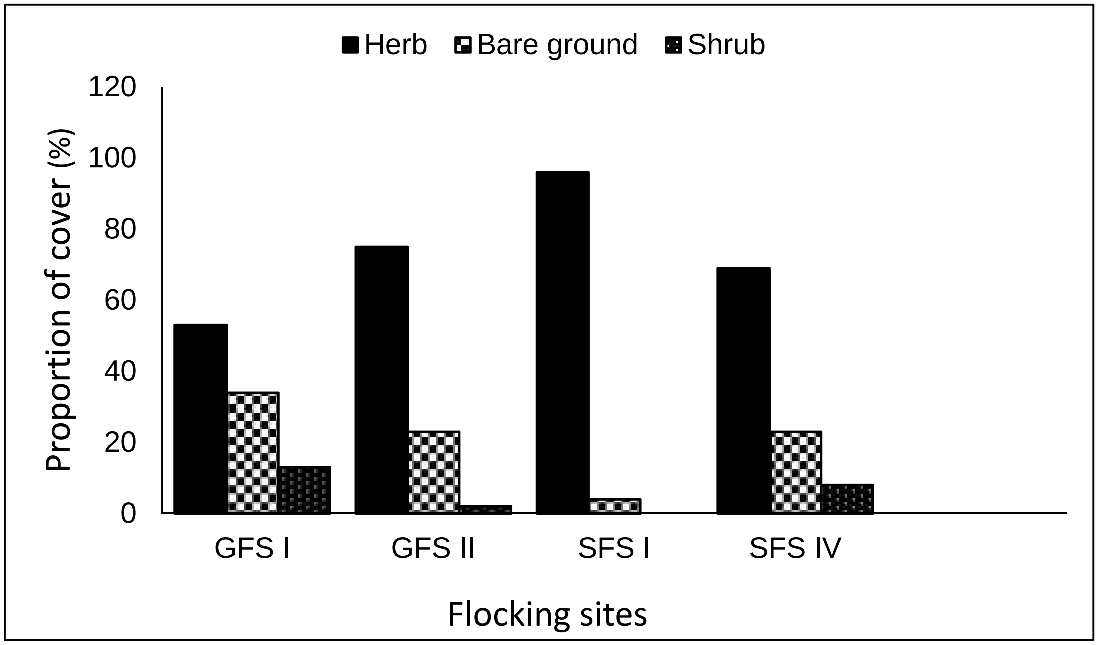
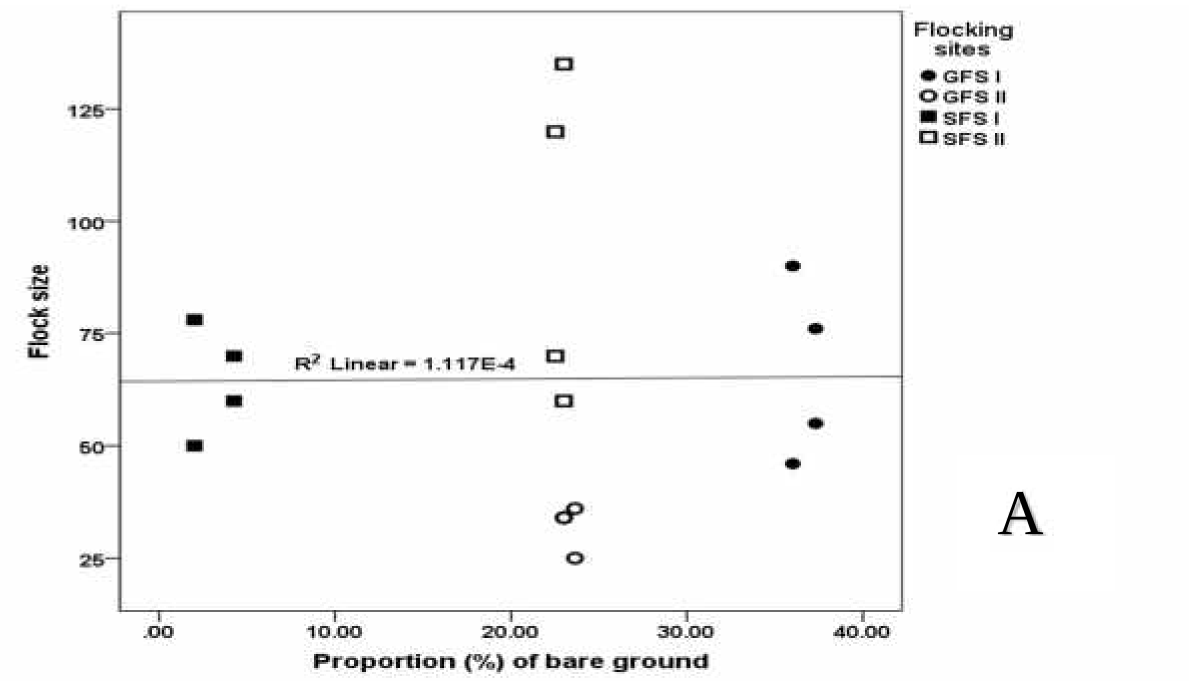
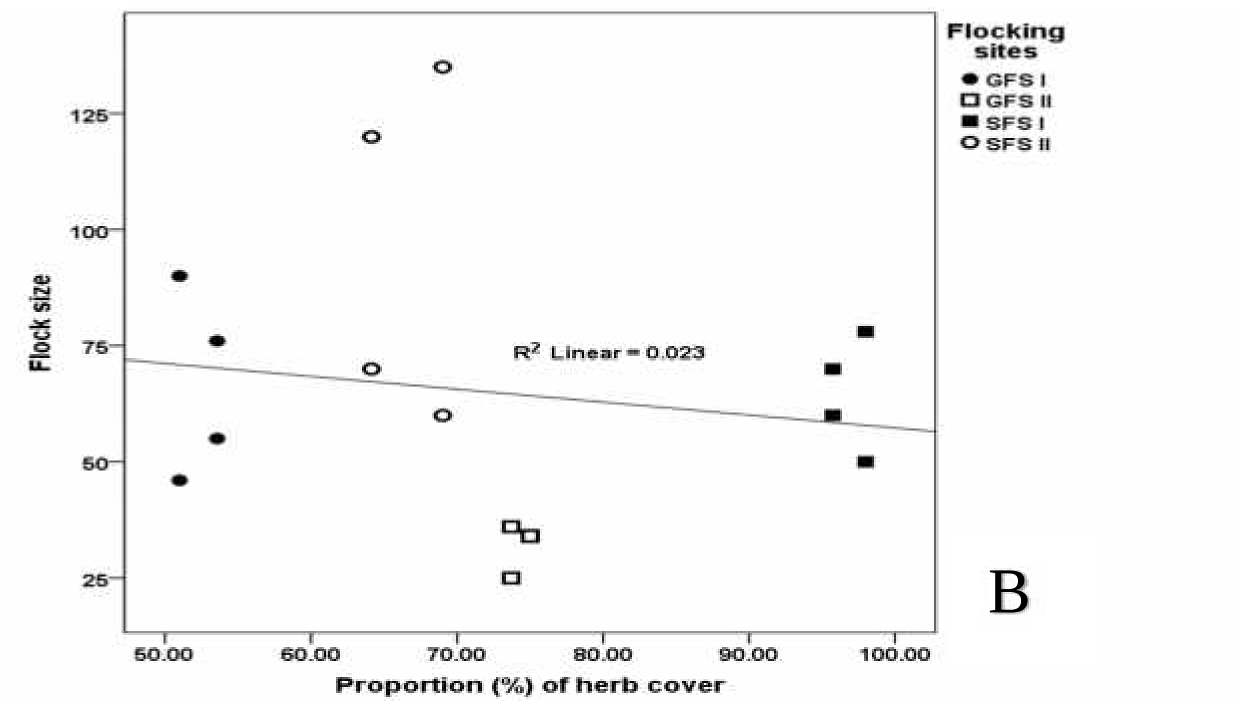


Figure 21. Ground cover of Guassa and simien flocking sites.

3.3.3.1. Flock size and ground cover

There was no significant correlation between flock size and proportion of ground cover of herbs ($r = -.152$, $p = .574$) bare ground ($r = .001$, $p = .969$) and shrub ($r = .357$, $p = .174$) (Fig. 22).


Flocking sites
GFS I
GFS II
SFS I
SFS II
125
100
75
50
25
Flock size
R² Linear = 1.117E-4
.00
10.00
20.00
30.00
40.00
Proportion (%) of bare ground
A


Flocking sites
GFS I
GFS II
SFS I
SFS II
125
100
75
50
25
Flock size
R² Linear = 0.023
50.00
60.00
70.00
80.00
90.00
100.00
Proportion (%) of herb cover
B

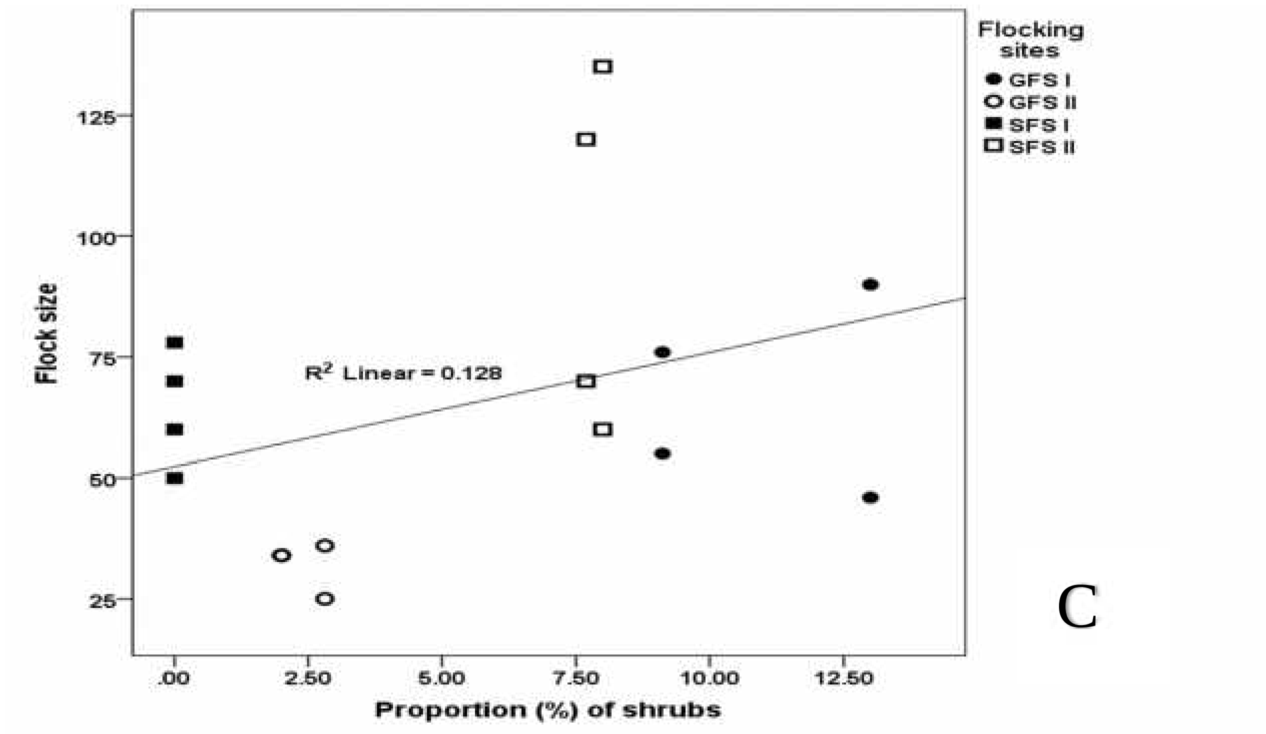


Figure 22. Correlation between flock size and cover of herbs (A) bare ground (B) shrubs (C) at the flocking sites (GFS I=Guassa flocking site I, GFS II=Guassa flocking site II, SFS I=Simien flocking site I, SFS IV=Simien flocking site IV).

3.3.4. Vertical stratification at the flocking sites

At GFS I, the vegetation heights <5 cm had relatively the highest proportion in the 1st (45.8%) and 2nd (49.5%) wet season followed by vegetation heights 21cm–30 cm with 15.7% and 14.5% in the 1st and 2nd wet seasons respectively (Fig. 23).

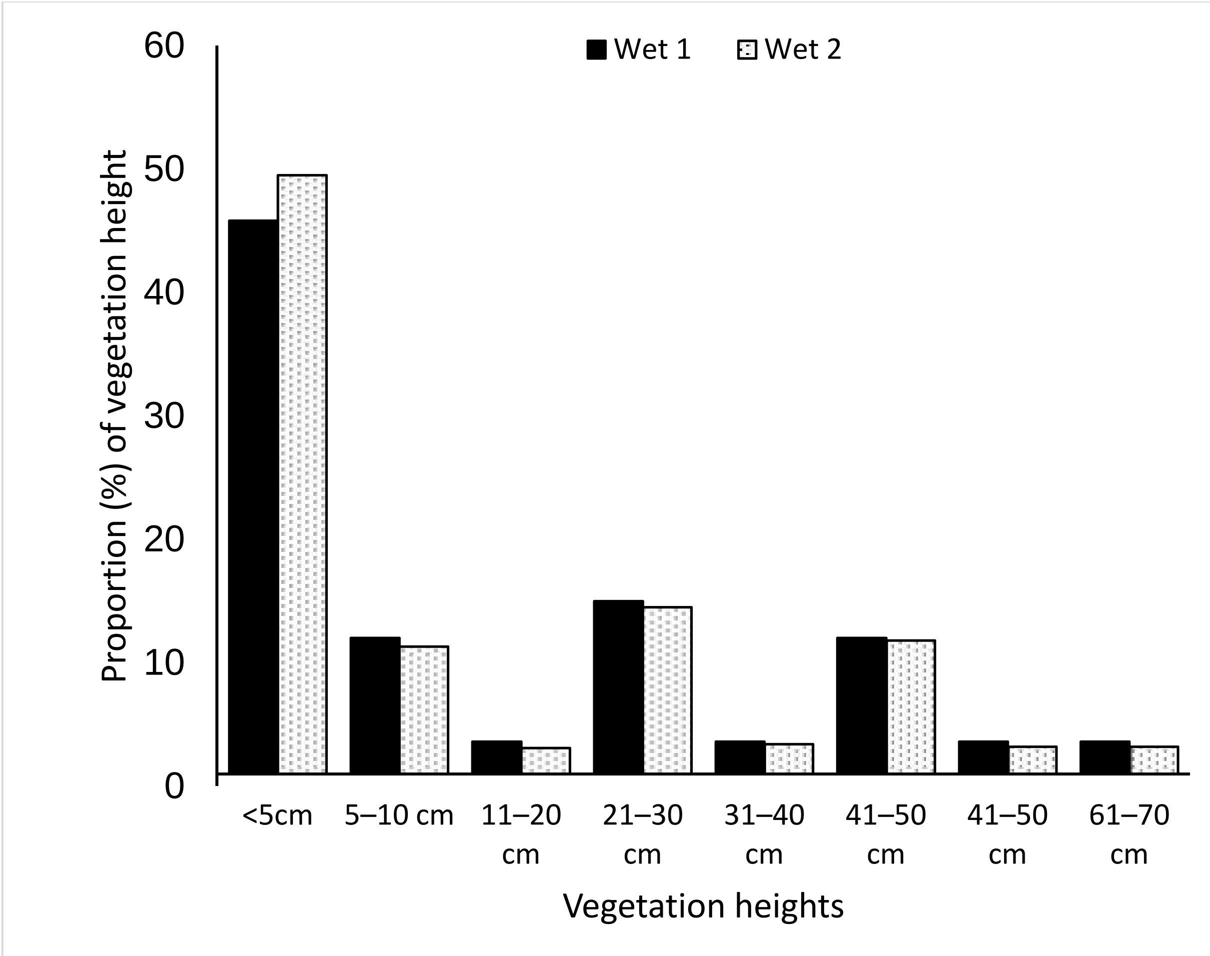


Figure 23. Proportion of vegetation height categories at GFS I.

At GFS II, the vegetation height (21-30 cm) had relatively the highest proportion (25.4%) and (25%) in the 1st and 2nd wet seasons, respectively, followed by vegetation heights <5 cm with 22.5% and 24.5% in the 1st and 2nd wet seasons respectively (Fig. 24).

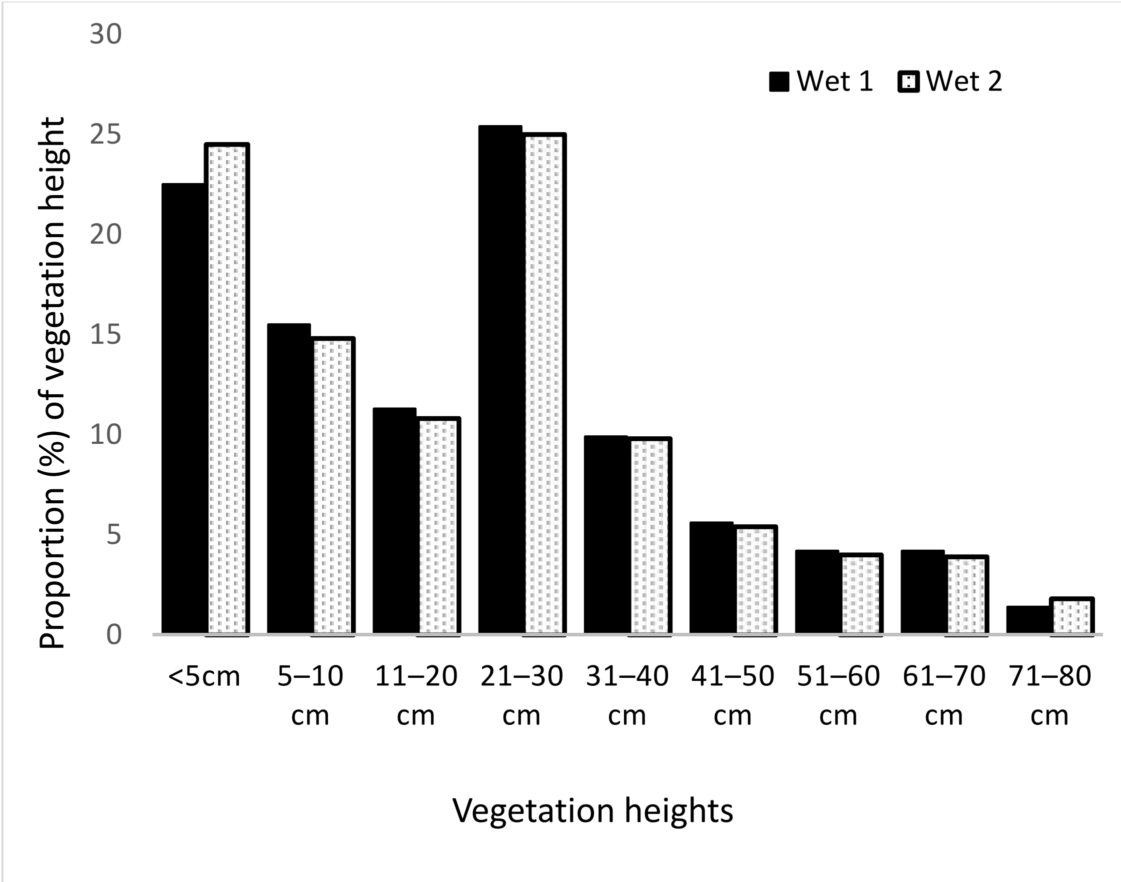


Figure 24. Proportion of vegetation height categories at GFS II.

At SFS I, the vegetation height <5 cms had relatively the highest proportion (77.2%) and (79.3%) in the 1st and 2nd wet seasons respectively; followed by vegetation heights 21-30 cm with 11.4% and 11.5% in the 1st and 2nd wet seasons respectively (Fig. 25).

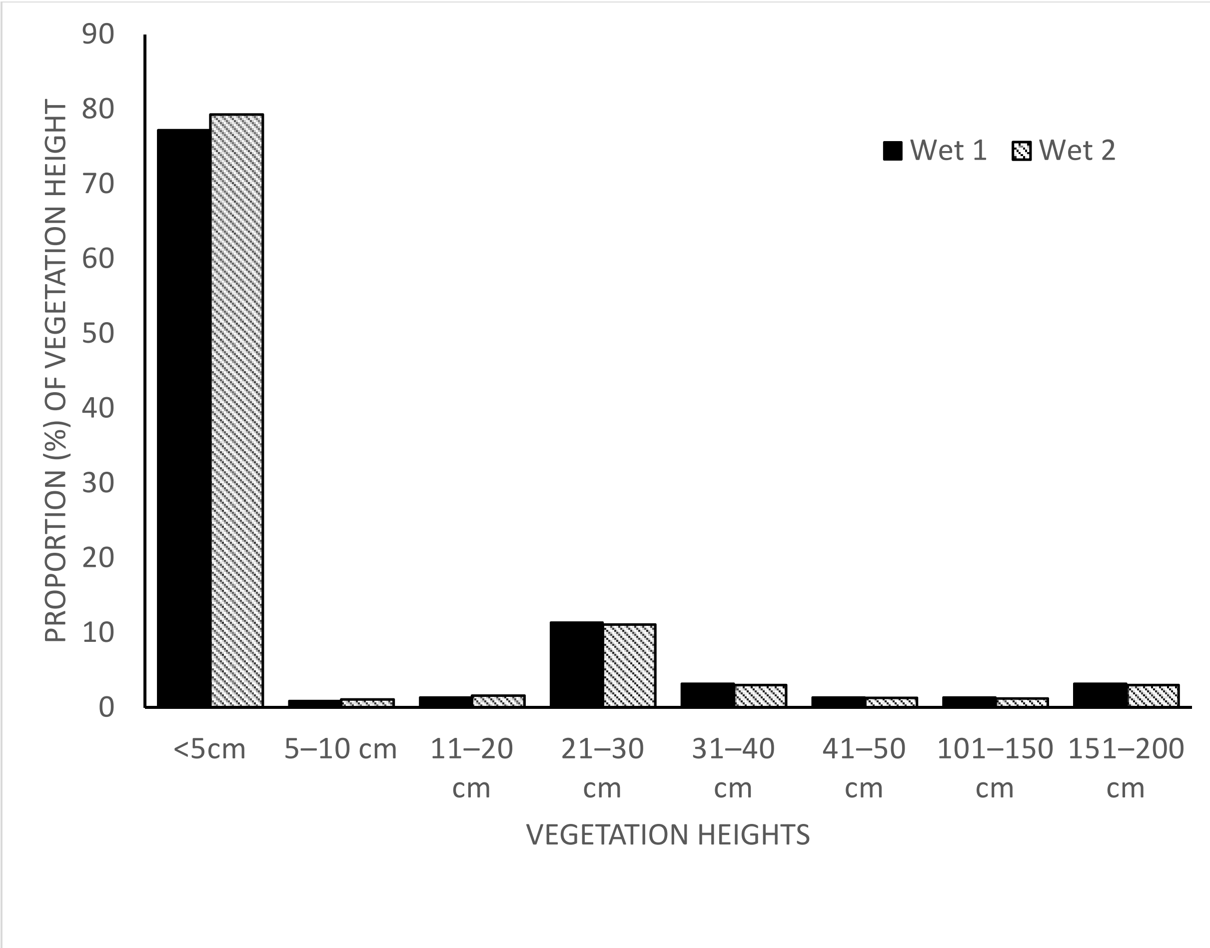


Figure 25. Proportion of vegetation height categories at SFS I.

At SFS IV, the vegetation heights <5 cms had relatively the highest proportions (22.9 %) and (23.6%) in the 1st and 2nd wet seasons respectively; followed by vegetation heights 5-10 cm with 21.2% and 21.4% in the 1st and 2nd wet seasons respectively (Fig. 26).

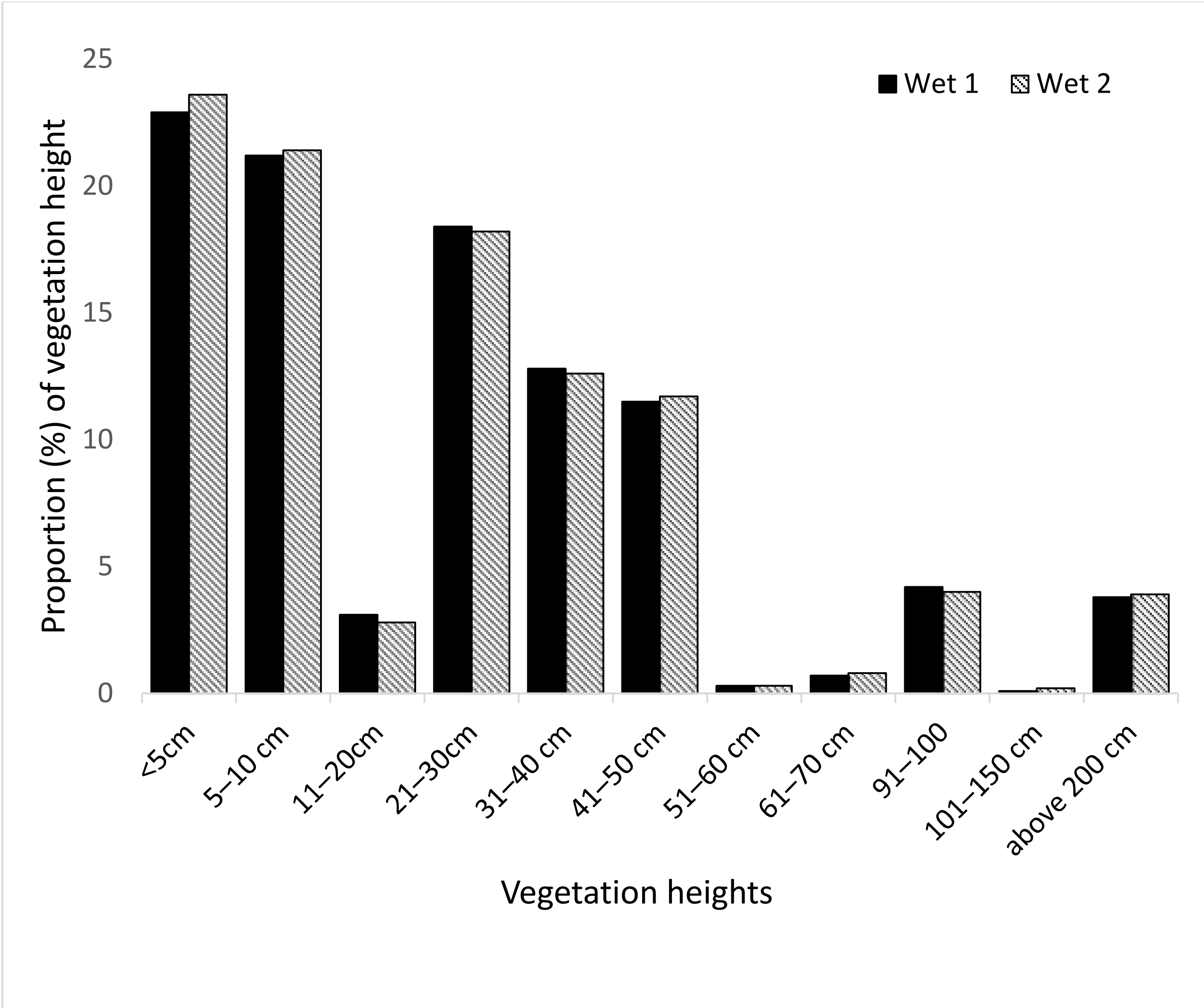


Figure 26. Proportion of vegetation height categories at SFS IV.

3.3.4.1. Flock size and vegetation height

The vegetation height category of < 30 cm constituted higher proportion (73%), followed by the vegetation height 31– 60 cms, representing 21%. Vegetation height above 60 cm had the least proportion in all the flocking sites (Table 12).

Table 12. Correlation of mean vegetation heights and mean flocking sizes (n= number of samples)

Flocking sites	Vegetation heights				Ovrall mean Flock sizes
	n=	< 30 cms (short)	31–60 cms (medium)	> 60 cms (long)	
GFS I	84	77. 1%	19.2%	3.6%	66.75±20
GFS II	80	74.7%	19.7%	5.6%	32.25±4.9
SFS I	231	75.1%	19.2%	5.7%	64.5± 12.2
SFS IV	678	65.6%	24%	9.7%	96.25±36.8

Flock size was negatively correlated with shorter vegetation height categories of <30 cm (r= -.535, p= 0.033). Flocking site SFS IV with relatively lower proportion (65.6%) of shorter vegetation heights had greater mean flock size (96.25). There was positive correlation between flock sizes and relatively medium vegetation heights (31-60 cms) (r= .589, p=0.016). The flocking site SFS IV with relatively higher proportion of medium vegetation heights had the highest flock size (96.25±36.8). There correlation was not significant between flock sizes and relatively taller vegetation heights (>60cms) (r= .476, p= 0.062) (Fig. 27).

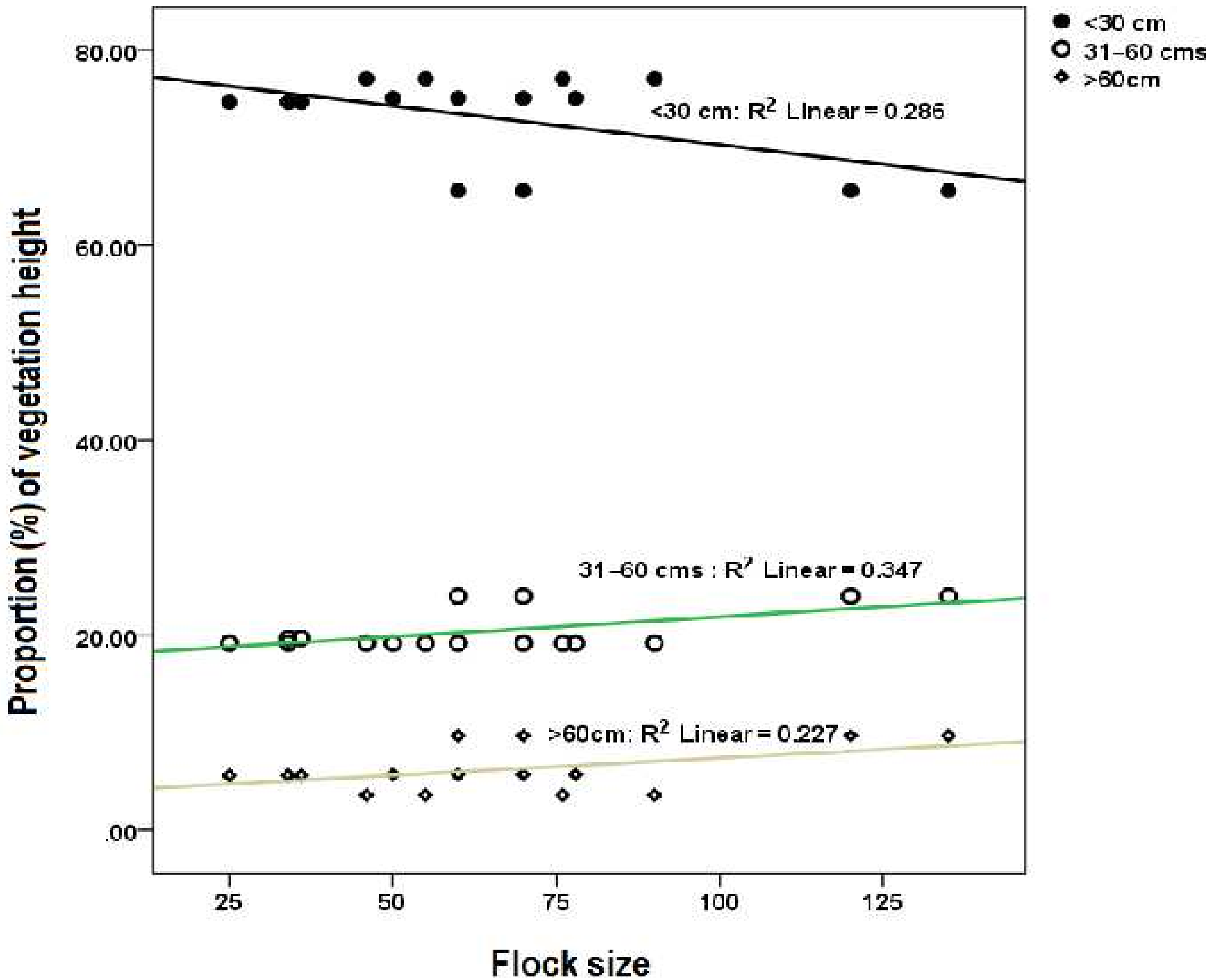


Figure 27. Correlation between flock size and the dominant vegetation height category (Short; <30cms), medium vegetation heights 30–60 cms and longer vegetation heights >60 cms.

The cliffs where Ankober Serin roosts and nests are characterized by rocks with crevices and interspersed herbs and shrubs almost with similar proportion (Fig. 28 A). In GFS I, the *Festuca* spp., *Helichrysum splendidum, Aeonium leucoblepharum*, *Festuca* spp. and *Erica* spp.; in GFS II, *Festuca* spp., *Aeonium leucoblepharum, Helichrisum splendidum, Kniphofia foliosa;* in SFS I, *Festuca* spp., *Kniphofia foliosa, Euphorbia* spp., *Aeonium leucoblepharum;* and in SFS IV, *Festuca* spp. *Helichrysum citrispinum* and *Rosularia simensis* were common. In all the flocking sites, *Trifolium* Spp. was also commonly observed in association with grasses. Ankober Serin's nest with four white eggs was seen on the cliff at GFS II (Fig. 28 B).

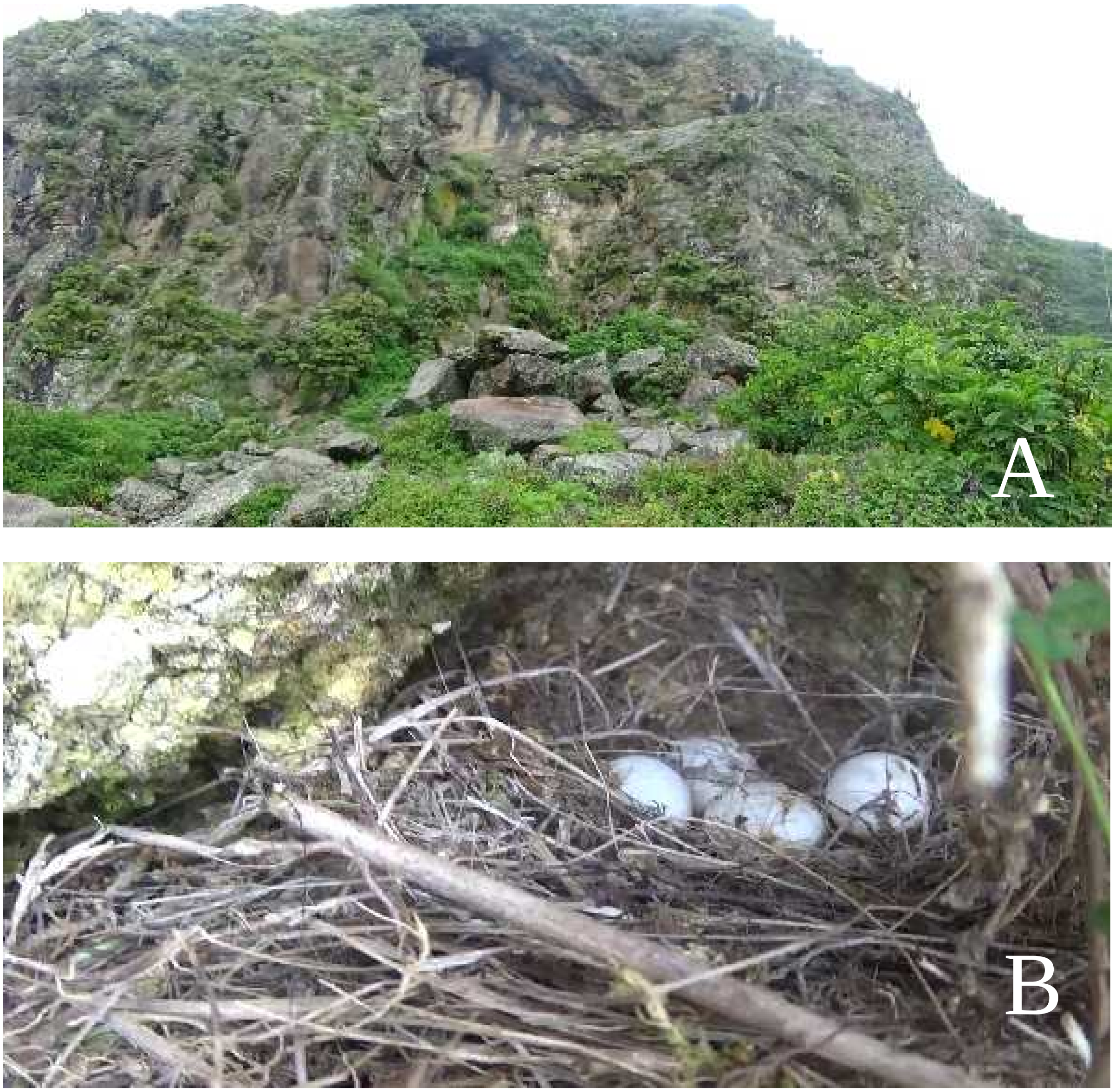


Figure 28. View of one of the roosting and nesting cliffs at GFS I (A) Ankober Serin nest with four eggs built in a crevice under a cliff at GFS II (B) (Photo: Abebayehu Desalegn, 2015).

3.4. Diet composition

Leaves dominated the wet season diet with mean proportion of 79.43±9.37% and 80.95±4.8 % at GCCA and SMNP, respectively. On the other hand, seeds were dominant in the dry season diet with mean proportion of 90.25±2.8% and 90.2± 2.6% at GCCA and SMNP, respectively (Table 13).

Table 13. Percentage proportion of diet items in fecal droppings of Ankober Serin at four flocking sites

Flocking sites	Proportion (%) of diet items in fecal droppings				
	Season	**Insect**	**Leaf**	**Moss**	**Seed**
GFS I	Wet 1	8.2	83.5	3.5	10.3
	Wet 2	10.5	65.6	2.1	22.4
	Dry 1	4.8	7.8	0.0	87.3
	Dry 2	1.2	10.4	0.0	88.4
GFS II	Wet 1	12.5	82.3	2.6	2.6
	Wet 2	5.1	86.3	0.6	8
	Dry 1	3.5	3.9	0	92.7
	Dry 2	1.6	5.8	0	92.6
SFS I	Wet 1	11.8	76	7	5.3
	Wet 2	11.7	79.1	4.6	4.6
	Dry 1	0.0	13.2	0.0	86.8
	Dry 2	4.7	5.8	0	89.5
SFS IV	Wet 1	11.9	81.3	6.8	0
	Wet 2	3.9	87.4	5.8	2.9
	Dry 1	0	5.1	2.2	92.7
	Dry 2	0	7	1.3	91.7

Wet season composition of the diet of Ankober Serin showed similar trend in both study areas with leaves, 79.43 ± 9.37 and 80.95±4.8; insects, 9.1 ± 3.179 and 9.8250 ± 4; seeds, 10.8250 ± 8.4 and 3.2 ± 2.4 and mosses, 2.2±1.2 and 6±1.1 at GCCA and SMNP, respectively. Similarly, during dry season, the diet of Ankober Serin was similar in both study areas with seeds >90 %, leaves > 6%, insects > .8% and insignificant proportion of mosses.

3.4.1. Sesaonal variation of diet composition in the two study areas

In GCCA, , all of the four diet items showed significant sesaonal variation between wet and dry seasons in their percentage proprtion of occurrence; seeds ($F_{1, 6}$= 324 p=0.000), leaves ($F_{1, 6}$= 219.842 p=0.000), insects ($F_{1, 6}$= 12.271 p=0.013), and mosses ($F_{1, 6}$= 13.140 p=0.011). Similaraly, there was significant sesaonal variation in the percentage proprtion of occurrence of the four diet categories at SMNP; seeds ($F_{1, 6}$= 2437.098 p=0.000), leaves ($F_{1, 6}$= 580. 246 p=0.000), insects ($F_{1, 6}$= 14.163 p=0.013), and mosses ($F_{1, 6}$= 13.140 p=0.011).

Flocks were seen to feed more frequently on the seeds of short turf of grasses mainly *Festuca* spp. (Fig. 29 A). The flock at SFS I was seen to forage the seeds of sorghum that was spilled on the road while passengers unloaded their sacs of sorghum from the truck crossing SMNP (Fig. 29 B).

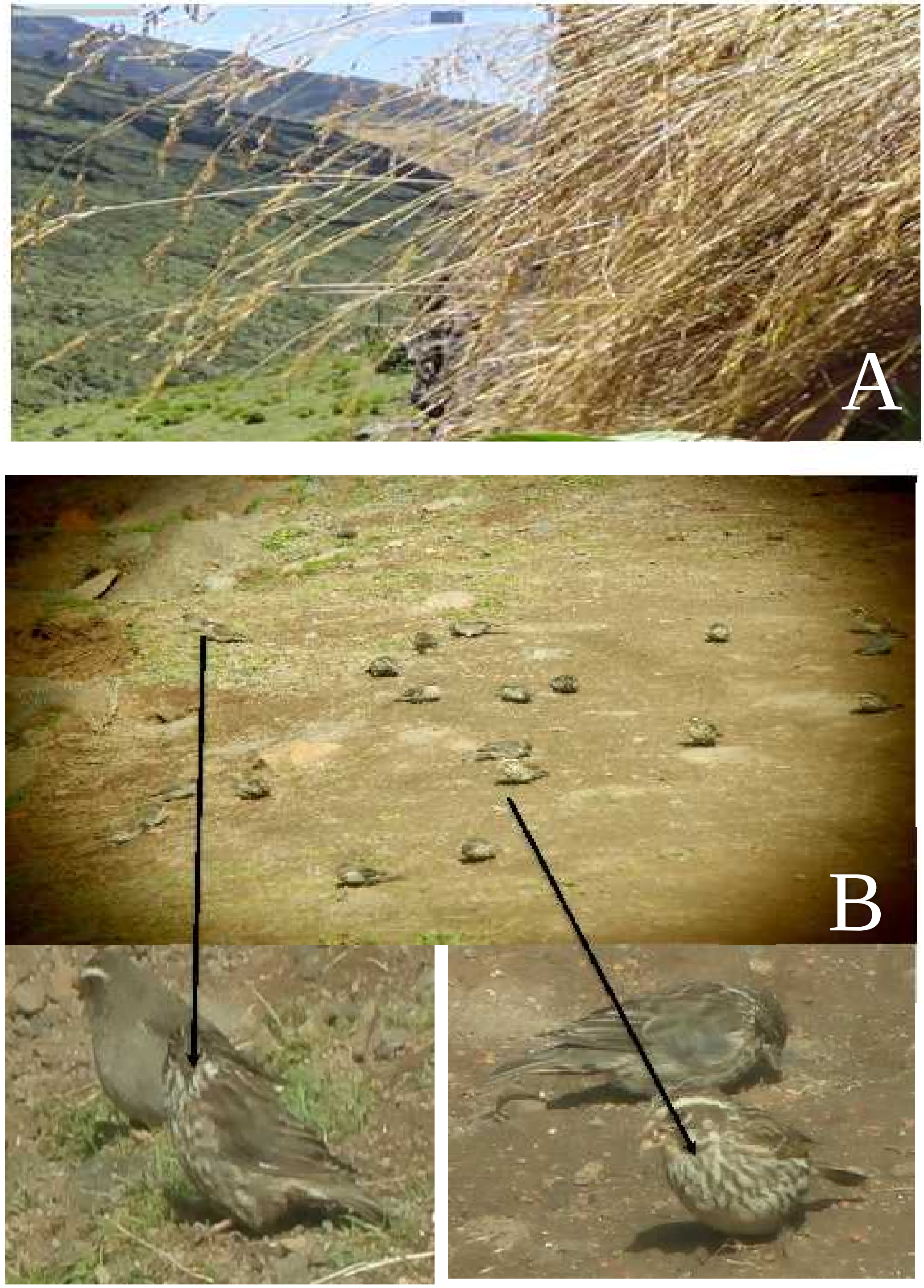


Figure 29. *Festuca* spp. which is frequently foraged by Ankober Serin at SFS I (A). Ankober Serin feeding on sorghum seeds in company with Brown-rumped *S. tristriatus* and Streaky Seed eater *S. striolatus* (B) (Photo: Abebayehu Desalegn 2015).

3.5. Genetic diversity

Five of the six markers provided successful PCR products for fragment analysis and one of those five markers showed no variation. The other four markers could be determined in almost all specimens. Sample numbers 001–205 were from Ankober Serin population of GCCA; and sample numbers 301–504 were from SMNP (Table 14).

3.5.1. Genic and genotypic differentiation

Genic and genotypic differentiation showed that both populations differ significantly in allele and genotype composition (Table 15), as three of the four markers showed significant differences, while only marker Cuμ04 was not differentiated between the two populations.

Genic differentiation

Three of the four markers i.e., Cuμ28, Ase43 and Ase42 exhibited significant differences between the populations ($p < 0.05$), while Cuμ04 was not differentiated ($p > 0.05$). When analysing all markers together, there was differentiation (the two populations have different sets of allels) ($p < 0.05$) (Table 15).

Genotypic differentiation

Three of the four markers i.e., Cuμ28, Ase43 and Ase42 showed significant difference (p-value > 0.05). When all were analyzed together the difference was significant (p-value < 0.05) (Table 15). Thus, the two populations differed markedly by their genotypes.

Table 14. Size of PCR products determined by fragment analysis (nd=not determined)

Sample No.	**Microsattelite markers**							
	Cuµ04	Cuµ04	Cuµ28	Cuµ28	Ase43	Ase43	Ase42	Ase42
sank001	129	133	152	152	240	240	403	403
sank002	133	133	154	154	240	246	nd	nd
sank003	131	131	154	160	246	248	389	389
sank004	131	131	154	154	240	246	389	409
sank005	131	131	152	154	242	248	387	403
sank006	129	131	154	154	242	242	387	387
sank101	129	131	154	154	nd	nd	387	387
sank102	131	133	154	154	240	240	403	403
sank104	131	133	154	160	240	242	401	403
sank201	129	131	154	154	240	240	403	403
sank202	131	133	154	160	240	240	387	387
sank203	131	133	154	154	240	252	403	403
sank204	131	133	152	152	240	246	389	405
sank205	131	133	154	160	240	240	387	387
sank301	131	133	154	154	242	242	403	403
sank302	129	131	154	154	240	240	385	403
sank303	129	133	154	154	240	240	403	403
sank304	131	133	154	154	242	242	403	403
sank305	133	135	154	154	238	246	385	403
sank306	133	133	154	154	232	240	387	387
sank401	123	129	154	154	Nd	nd	385	385
sank402	129	131	154	154	238	238	387	387
sank403	131	133	154	154	242	242	403	403
sank404	131	133	154	154	234	240	385	385
sank405	129	131	154	154	234	240	385	385
sank406	133	133	154	154	234	242	385	385
sank501	129	131	154	154	234	240	385	403
sank502	129	133	154	154	234	242	385	385
sank503	127	129	154	154	240	240	387	387
sank504	131	131	154	154	242	242	389	389

3.5.2. Hardy-Weinberg- equilibrium

The Hardy-Weinberg equilibrium showed the following results:

i. Using the Chi-square test, the molecular markers Cuµ28, Ase42 showed significant deviation from Hardy-Weinberg equilibrium with in the population at GCCA.

ii. Using the Chi-square test, the molecular markers Ase42, Ase43 showed significant deviation from Hardy-Weinberg- equilibrium with in the population at SMNP. Cuμ28 could not be inferred, because there was a single allele.

iii. Hardy-Weinberg equilibrium of all specimens showed similar results as the Chi-square tests. So, they were having major deviations between themselves.

Thus, in both populations, two markers- Cuμ28 and Ase42 (GCCA); and Ase43 and Ase42 (SMNP) were not in equilibrium.. However, in the SMNP population, Cuμ28 was uniformly homozygous for just one allele, therefore Hardy-Weinberg equlilibrium cannot be tested statistically (Table 15).

Table 15. P-values of statistical tests for single markers and all markers combined (Genic differentiation and genomic differentiation according to exact G test and Hardy-Weinberg exact test, probabilities according to Chi-square method).

Locus	Genic differentiation	Genotypic differentiation	Hardy-Weinberg equilibrium Pop1	Hardy-Weinberg equilibrium Pop2	Hardy-Weinberg equilibrium all pop.
All loci	3.26e-007	9.11e-005	0.0017	0.0000	0.0000
Cuμ04	0.31795	0.26165	0.8553	0.6693	0.8918
Cuμ28	0.00038	0.00147	0.0486	No inf.	No inf.
Ase43	0.00182	0.015	0.2138	0.0006	0.0013
Ase42	0.00067	0.01899	0.0005	0.0000	0.0000

4. Discussion

4.1. Flock size

The significantly higher number of individuals of Ankober Serin per five flocking sites in SMNP than GCCA may be attributed to the presence of extensive and undisturbed foraging grounds. Though the observed minimum flock size of 15 individuals per flock was consistent with the previously known minimum flock sizes of 13, the observed flock size of maximum 140 individuals per flock was higher than the flock size of 100 individuals reported by various researchers (EWHNS 1996; Anteneh Shimelis1999; Ash and Atkins 2009; Vivero 2001).

Similarly, the significantly higher flock size at SMNP than GCCA could be attributed to the differences in the degree of disturbance and difference in the size of the foraging ground. The results of the present study showed that the highest average flock sizes were counted in SFS V; and the lowest at GFS II. This may be similarly attributed to the degree of disturbance and habitat size. The overall mean flock size during the dry seasons was higher than the wet seasons. Such increase in flock size during the dry season may be due to the accessibility of seeds produced after late wet seasons. Yang *et al.* (2015) revealed that food availability and abandance affect flock patterns of foraging birds. Raitt and Pimm (1976) also suggested that density of grassland birds increased after late summer rains. The dry season also coincides with the breeding season of Ankober Serin which can also explain the observed high flock size (EWNHS 1996). Newton (2003) indicated that the annual cycles of most birds fall into two distinct phases: a breeding summer season, when numbers increase because reproduction exceeds mortality; and a non-breeding winter season when numbers decline because of mortality (Newton, 2003). Numbers therefore reach a seasonal peak immediately after one breeding season. Most bird species can be counted most easily when they are breeding.

4.2. Habitat disturbance

The low flock size observed at more disturbed sites might have resulted from reduction of seed bearing grasses which are the preferd food sources of Ankober Serin. Though, in some studies, higher degree of disturbance together with abundant food was associated with increased number of flock sizes (Yang *et al.*, 2015), persistent disturbances could make foraging grounds much more deteriorated or less resourcefull. Cresstwell (2008) also pointed out that extreme and persistent disturbance can exclude birds from their feeding habitats by reducing the availability of food. Bird population sizes can be affected if human presence deters birds from fully exploiting resources. Thus, such disturbances can have the same effect as natural predation risk (Newton 2013).

4.3. Habitat analysis

There was significant correlation between flock size and *Festuca* spp. dominated flocking site (SFS IV). In this undisturbed habitat, the available seed bearing grasses can attain their full developmental stages to the production of seeds. In addition, the relatively larger foraging areas guarantee abundance of seeds. Therefore, the higher flock size, especially during the dry seasons in this community may be due to availability of abundant food resources. On the other hand, flock size was lower at the *Cynodon* spp. dominated foraging ground (GFS II). This community is a highly trampled and grazed habitat or with high human interferences. It is also the most fragmented site. This result confirms that habitat fragmentation and alteration are potential threats to the survival of Ankober Serin (Wilcove *et al.* 1986). Sutherland *et al.* (2004) stated that vegetation composition may directly influence food supply, for example, by providing suitable seeds or palatable grass species for the animals depending on them.

In the present study no consistent relation between vegetation diversity and flock size. Some studies also indicated that bird richness and abundance were not related to vegetation richness (Silva *et al.* 2015). However, other studies showed positive relationship between plant species diversity and bird richness and abundance (Joshi *et al.* 2012). The occurrence of specific plant species may be more important than species diversity (this requires further investigation) to Ankober Serin.

The results of the study showed that Ankober Serin prefers herbs, more specifically grasses dominated habitat. Ash (1979) also pointed out that Ankober Serin alights on no other vegetation than grasses. It also prefers interspersed rocks where it can temporarily perch and feed on the relatively moist ground under the rocks (Fig. 30 A and B). Rocks may also serve as temporary hiding places (Fig. 30 C). Bare earth may also increase access for birds to soil invertebrates (Perkins *et al.* 2000) and surface-living insects, while a dense litter layer will reduce it. Rocks may also provide nest-sites and look-out points as seen in figure 30 A and B (Green *et al.* 1990). It is thus correct to characterize Ankober Serin as one of the Ethiopian highland species of birds inhabiting rocky habitats (Ash 1979).

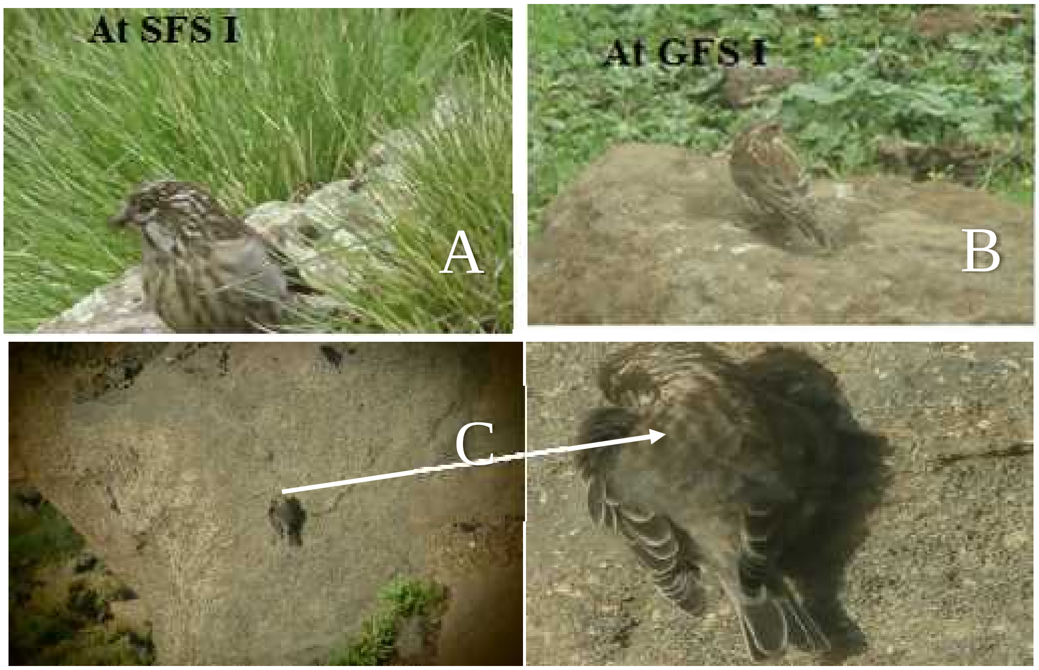


Figure 30. Ankober Serin perching on rocks (A and B); concealing itself on the side of a rock surface (C) (Photo: Abebayehu Desalegn 2015)

As the results have shown, short vegetation heights that are <30 cm had higher proportion in all the foraging sites of Ankober Serin. Of these the height category of <5 cm had highest proportion in most of the foraging grounds of the flocking sites. This suggests that Ankober Serin prefers a greater proportion of short and open vegetaion cover instead of tall and dense vegetation cover. EWNHS (1996) and J. Vivero (2001) also noted that Ankober Serin prefers short-grazed pasture. With in the short and open vegetaion cover, the occurrence of relatively greater proportion of medium (31–60 cms) and taller (> 60 cm) vegetation height was more prefered.

The results on the visual assessment of the nesting and roosting cliffs of Ankober Serin revealed that the cliffs are characterizeed by rocks with crevices, interspersed herbs and shrubs almost in similar proportion. In addition, one nest with four eggs was also observed, though, it is known that Ankober Serin's clutch-size is three (EWNHS 1996). The steep, rugged terrain of Ankober Serin's cliffy habitat offers some protection to the species thus used as their hiding place (EWNHS 1996; Vivero 2003). Whenever there was disturbance in their habitats, their flight was immediately to the nearby cliff. The flock usually had more than one alternative cliff faces. Cliff is natural roosting habitat of Ankober Serin. Cliffs reserve natural habitat structure and composition. Ankober Serin nests mainly on cliffs (Ash 1979), and it is rarely found where cliffs are lacking. Most bird species are sensitive to disturbances during their breeding season. Nests, eggs, chicks, and adults in the nest are vulnerable to predators (Sutherland *et al.* 2004), and hence vertical cliffs are relatively safer for nesting, laying eggs and raising the chicks. Thus, it is possible to suggest that roosting and nesting cliff, not the foraging ground that is much more crucial to the persistence of this species. Due to the unique physical features, the Simien Mountains are noted as refugia to rare and endemic animals (Puff and Sileshi 2001).

4.4. Diet composition

The results of the present study show that during the dry season, Ankober Serin feeds largely on seeds of grasses that are abundant during the season in the flocking sites. The availability of seeds gradually declines due to germination and consumption (Newton 2013). During the wet season, Ankober Serin feeds mainly on the leaves of grasses. Vivero (2004) also pointed out that Ankober Serin is mainly a seed eater and feeds on grass, most commonly in flock and sometimes individually. The results also suggest that during scarcity of seeds, Ankober Serin switches to foraging predominantly on blades of grasses. Hoyo *et al.* (2007) indicated that seeds are consumed throughout the year, supplemented at times by leaves, shoots and other green matters by seed eating birds. Some bird species prefer to eat seeds in winter, but can also subsist on green grass (Potts 2012). Such dietary switching, corresponding to temporal variations in food resource availability, was also seen in the diet composition of the endemic Madeira Laurel Pigeon (*Columba trocaz*) (Oliveira *et al*. 2002). Ankober Serin also prefers a newly tilled farmland, may be in search of weeds and insects (visual obesrvation) as Larke *et al*. (2003) have also noted that grasses and arable weeds are of critically important in the diet of birds foraging in open grassland and farmland habitats. Mosses contributed a small proportion to the diet. Lack of variation in diet composition between the flocking sites shows that Ankober Serin probably has the same mode of feeding habit and dietary rigidity regardless of locations.

4.5. Feeding observations

Ankober Serin was seen to feed on seeds of sorghum opportunistically. Sorghum is not cultivated in the highlands. This habit of the birds shows that they have the potential of dietary plasticity to adapt to conditions of food scarcity. Gaston (1994) and Malcolm *et al*. (2002) have stated that modern fragmented landscapes and rapidly changing climatic conditions favour well-developed dispersal abilities and opportunistic species. Nonetheless, the vulnerability of these species is not restricted to only food availability instead its survival is also affected by factors such as nesting sites and ability to avoid predators.

4.6. Genetic diversity

Results of the present study showed that the two populations of Ankober Serin at GCCA and SMNP have differentiated from each other, suggesting a long history of separation.

However, the shared alleles between the two populations suggest ongoing gene flow and that the two populations still belong to the same species.

The long history of separate evolution that caused significant differentiation between the two populations may be the physical features of the study areas as these locations are separated by mountains, valleys and plains as well as the separation distance which is more than 360 kms. This assumption may be in line with the studies made on house sparrow populations on the coast of Norway where populations are separated by fjords and mountains that showed significant variation (Bjordal *et al.* 1986). Significant differentiation was also observed between populations of a migratory songbird, *Limnothlypis swainsonii* even though there were no steep environmental gradients or obvious barriers to gene flow between southern Louisiana and eastern Arkansas that would promote differentiation (Winker *et al.* 2000).

Due to the shared alleles between the two populations the current study indicates gene flow is ongoing. This may also happen by a stepping-stone pattern of small migration distances in a fairly homogenously distributed population as in the case of Finnish house sparrow (Kekkonen *et al.* 2011). In addition to SMNP and GCCA, Ankober Serin is distributed in the ecologically similar habitats throughout the highland massif of the Amhara Regional State (Vivero 2003). Such distribution might have favoured the stepping-stone pattern of small migration distances for the ongoing gene flow. Molecular studies on the Liben (Sidamo) Lark and Archer's Lark showed that the two populations were not only genetically very similar, but did not even form separate monophyletic groups. This is suggestive of recent gene flow between the Horn of Africa populations, or very recent separation (Spottiswoode 2012).

All the above explanations suggest the significant differentiation and gene flow between the two populations of Ankober Serin in SMNP and GCCA. Nevertheless, the factors affecting the genetic diversity of populations of Ankober Serin should be studied. Thus, studies that combine phylogenetic approaches with analyses of gene flow, as well as the ecological factors promoting the significant differentiation and gene flow on the populations of Ankober Serin should be conducted.

Results of the present study showed that there was significant deviation from the Hardy–Weinberg equilibrium. In many studies of population genetics, explanations for the lack of agreement with Hardy–Weinberg equilibrium include selective mating, population sub-structuring, low levels of polymorphism, limited population (shortage of samples), and restricted gene flow. Though evidences for the deviations are not available, causes for significant deviations from this equilibrium of Ankober Serin populations might lie in the limited population size or restricted gene flow in this species.

4.7. Conclusion

The present research recorded the lowest flock size as 15 individuals per flock which is comparable with other reports that recorded 13 individuals per flock; while the highest record of this study was up to 140 individuals per flock which was slightly greater than the previous record up to 100 individals per flock. The degree of habitat disturbances is between medium and high. Therefore, the habitat is still threatened and declining. It will be worse if the cliffs are highly disturbed. Some of the cliffs are within human reaches. The other inaccessible cliffs have shielding effect that helped the species to survive so far.

The habitats of Ankober Serin are patches of open grassland and rocky habitats with nearby roosting and nesting cliffs. Most of the foraging grounds of the species are dominantly covered by herbs ollowed by bare earth with shorter vegetation heights. Large portions of the foraging sites of this species are disturbed sites. The highest number of birds were recorded in naturally open grassland habitats with undisturbed cliffs. Moist and vegetated vertical cliffs are critical habitats for nesting and roosting of the species. As the wet and dry season data showed, the species was present throughout the year in the study areas.

Ankober Serin is mainly a seed eater. During the dry season, seeds predominate the diet while during the rainy season seeds are scarce and the diet shifts toleaves and insects.

The two populations of Ankober Serin at GCCA and SMNP are genetically differentiated from each other, suggesting a long history of evolutionary separation. Nevertheless, there are a lot of shared alleles between the two populations suggesting ongoing gene flow. It can be confirmed that the two populations belong to the same species. Deviations from

Hardy-Weinberg equilibrium in two markers in each of the two populations may be explained by recent migration events between each other or other neighboring populations.

4.8. Recommendations

- Habitat of Ankober Serin is restricted to such a small range and affected by human related disturbances. Therefore, conservation of herbs and shrubs covering the foraging grounds and hanging on the nesting cliffs is essential conservation action.
- Maintenance of the open habitats, the *Festuca* spp. and other grasses and herbs is important. The local community should be encouraged to manage the habitat by controling the grazing and the timing of grass cutting in the habitat of Ankober Serin.
- Continuous monitoring of Ankober Serin population trend needs to be carried out at regular intervals to plan necessary interventions.

- Further studies are recommended to understand the causes of significant genetic differentiation and deviations from Hardy—Weinberg equilibrium of these two populations of Ankober Serin.

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Appendices

Appendix 1. IUCN categories of Ethiopian endemic birds (Source Vivero, 2001).

№	Common names	Scientific names	IUCN Category
1	Spot-breasted Lapwing	*Vanellus melanocephalus*	LR nt
2	Abyssinian Catbird	*Parophasma galinieri*	LR nt
3	Abyssinian Long-claw	*Macronyx flavicollis*	LR nt
4	Abyssinian Woodpecker	Dendropicos abyssinicus	LR nt
5	Ankober Serin	*Serinus ankoberensis*	VU
6	Black-headed Siskin	*Serinus nigriceps*	LR lc
7	Degodi Lark	*Mirafra degodiensis*	VU
8	Ethiopian Bush-Crow	*Zavattariornis stresemanni*	VU
9	Harwood's Francolin	*Francolinus harwoodi*	VU
10	Nechisar Nightjar	*Caprimulgus solala*	VU
11	Prince Ruspoli's Turaco	*Tauraco ruspolii*	EN
12	Salvadori's Serin	*Serinus xantholaema*	VU
13	Sidamo Long-clawed Lark	*Heteromirafra sidamoensis*	VU
14	White-tailed Swallow	*Hirundo megaensis*	VU
15	Yellow-fronted Parrot	*Poicephalus flavifrons*	LR nt
16	Yellow-throated Serin	*Serinus flavigula*	EN

IUCN categories: CR=Critical, EN=Endangered, VU=Vulnerable, LR cd=Low Risk conservation dependent, LR nt=Low Risk near threatened, LR lc=Low Risk least concern.

Appendix 2. Habitat, Altitudnal ranges and Distribution of endemic birds of Ethiopia.

(Source Vivero 2001).

Scientific name	Habitat	Altitude	Distribution
Francolinus harwoodi	Open habitat in highlands	2000-2500	Restricted to western central highlands
Vanellus melanocephalus	Open habitat in highlands	1800-4100	Northern & central high lands
Poicephalus flavifrons	Forest & Woodland	600-3800	West, southeast highlands & west lowlands
Tauraco ruspolii	Forest & Woodland	1300-1900	Borena zone
Caprimulgus solala	Open savannah	1200-1400	Nechisar National Park
Dendropicos abyssinicus	Forest & Woodland	1600-3000	Throughout the highlands
Mirafra degodiensis	Open savannah	320-350	Borena zone, lowlands
Heteromirafra sidamoensis	Open savannah	1450	Borena zone, lowlands
Hirundo megaensis	Open savannah	1000-1700	Borena zone, lowlands
Macronyx flavicollis	Open habitats	1200-4100	Throughout the highlands & lowlands
Parophasma galinieri	Forest	2400-3000	Central & southern highlands
Zavattariornis stresemanni	Open savannah	1500-2000	Borena zone, lowlands
Serinus nigriceps	Open habitats	1800-4200	Throughout the highlands
Serinus ankoberensis	Rocky habitats	2800-4000	Ankober, Simien
Serinus flavigula	Open savannah	1400-1500	Ankober lowlands
Serinus xantholaema	Open & Rocky habitats	1500-1700	Harar, Bale & Borena zones

Appendix 3. Primer sequences

(Fluorescent dyes Cy5 and Cy5.5. were added at 5-end of forward primers; Gf08 was not used for fragment analysis due to bad PCR performance in these samples)

Ase42

Forward: Cy5-CATGGGTAGGTTGGGATGTC

Reverse: AGGTGAGGGTATGCAAACATG

Ase43

Forward: Cy5-ATTGTGTGGGATTTGCAT

Reverse: TTGCTGTGCAGTTTGCTTTT

Ase48

Forward: Cy5.5-TTTATTTCCTGGACTGGAACAATC

Reverse: GAACATTGGGCTACTGGGC

Cuµ04

Forward: Cy5-AATTGCATAAATGTGATCCAC

Reverse: AAATGAAATGTGGTAGAATTCC

Cuµ28

Forward: Cy5.5-GAGGCACAGAAATGTGAATT

Reverse: TAAGTAGAAGGACTTGATGGCT

Gf08

Forward: Cy5-TGGGAGAGCAAGGTGGGAACAG

Reverse: TGGAGTGGTGATTAAACCAGCAGG

Appendix 4. Raw data file as input for Genepop.

(file starts next line)

Serinus ankoberensis Ethiopia

CU04

CU28

AS43

AS42

pop

sank001	,	129133	152152	240240	403403
sank002	,	133133	154154	240246	000000
sank003	,	131131	154160	246248	389389
sank004	,	131131	154154	240246	389409
sank005	,	131131	152154	242248	387403
sank006	,	129131	154154	242242	387387
sank101	,	129131	154154	000000	387387
sank102	,	131133	154154	240240	403403
sank104	,	131133	154160	240242	401403

sank201	,	129131	154154	240240	403403
sank202	,	131133	154160	240240	387387
sank203	,	131133	154154	240252	403403
sank204	,	131133	152152	240246	389405
sank205	,	131133	154160	240240	387387
Pop					
sank301	,	131133	154154	242242	403403
sank302	,	129131	154154	240240	385403
sank303	,	129133	154154	240240	403403
sank304	,	131133	154154	242242	403403
sank305	,	133135	154154	238246	385403
sank306	,	133133	154154	232240	387387
sank401	,	123129	154154	000000	385385
sank402	,	129131	154154	238238	387387
sank403	,	131133	154154	242242	403403
sank404	,	131133	154154	234240	385385
sank405	,	129131	154154	234240	385385

sank406	,	133133	154154	234242	385385
sank501	,	129131	154154	234240	385403
sank502	,	129133	154154	234242	385385
sank503	,	127129	154154	240240	387387
sank504	,	131131	154154	242242	389389

(end of file before this line)

Appendix 5. Data collection sheet for vegetation composition and diversity

Date___________ **flocking site _____________**

Study area______________

Sampling unit	coordinates	elevation	Plant species	Habit
1				
2				
3				

Appendix 6. Data collection sheet for proportion of cover in each sampling unit

Date___________ **flocking site _____________**

Study area______________

Sampling unit	coordinates	elevation	% of herb	% of shrub	% of bare
1					
2					
3					

Appendix 7. Data collection sheet for vegetation heights

Date___________ **flocking site _____________**

Study area______________

Sample no	Vegetation heights													
	<5 cms	5–10 cms	11–20 cms	21–30 cms	31–40 cms	41–50 cms	51–60 cms	61–70 cms	71–80 cms	81 – 90 cms	91–100 cms	101–150 cms	151–200 cms	>200 cms
1														
2														
3														
4														
5														
6														
7														

Appendix 8. Pellet collection sheet for diet composition study

Date	Time	Sample no	Site of collection	Remark

Appendix 9. Scientific names, family and habit of the plant species in both study areas.

Species names	**Family**	**Type**
Festuca macrophylla Hochst. ex A. Rich.	Poaceae (Gramineae)	Shrub
Lobelia rhyncopetalum	Lobeliaceae	Herb
Myosotis vestergrenii	Boraginaceae	Herb
Rumex spp	Polygonaceae	Herb
Satureja simensis (Benth.) Briq.	Lamiaceae	Herb
Plectranthus lanuginosus (Hochst. ex Benth.)	Lamiaceae	Herb
Primula verticillata Forssk. ssp.	Primulaceae	Herb
Ranunculus Schimperi	Ranunculaceae	Herb
Sagina afroalpina Hedberg	Caryophyllaceae	Herb
Salvia merjamie Forssk.	Lamiaceae	Herb
Satureja abyssinica (Benth.) Briq.	Lamiaceae (Labiatae)	Herb
Satureja pseudosimensis Brenan	Lamiaceae (Labiatae)	Herb
Satureja punctata (Benth.) Briq.	Lamiaceae (Labiatae)	Herb
Senecio nanus Sch.Bip. ex A.Rich.	Asteraceae	Herb
Thymus schimperi Ronniger	Lamiaceae	Herb
Thymus serrulatus Hochst. ex Benth	Lamiaceae	Herb
Trifolium spp. Fresen	. Fabaceae (Leguminosae)	Herb
Urtica simensis Hochst. ex A. Rich	Urticaceae	Herb

Species names	**Family**	**Type**
Agrostis quinquesta (Steud.) Hochs	Poaceae (Gramineae)	Herb
Agrostis sclerophylla C.E.Hubb	Poaceae (Gramineae)	Herb
Alchemilla (Aphanes) bachitii	Rosaceae	Herb
Aeonium leucoblepharum A. Rich.	Crassulaceae	Herb
Alchemilla pedata	Rosaceae	Herb
Andropogon spp.	Poaceae (Gramineae)	Herb
Bidens macroptera (Sch.Bip. ex Chiov.) Mesfin	Asteraceae	Herb
Carduus schimperi	Asteraceae (Compositae)	Herb
Carex spp.	Cyperaceae	Herb
Cerastium octandrum Hochst	Caryophyllaceae	Herb
Crassula alba Forssk.	Crassulaceae	Herb
Crassula Schimperi	Crassulaceae	Herb
Cynodon spp	Poaceae (Gramineae)	herb
Cynoglosum coeruleum Hochst. Ex A. DC	Boraginaceae	Herb
Dianthoseris schimperi	Asteraceae (Compositae)	Herb
Euphorbia schimperiana Scheele	Euphorbiaceae	Herb
Euryops pinifolius A.rich	Asteraceae (Compositae)	Herb
Galium simense. Fresen.	Rubiaceae	Herb
Haplocarpha schimperi (Sch.Bip.) Beauv	Asteraceae	Herb
Helichrysum splendidum	Asteraceae (Compositae)	Shrub
Hypericum revolutum	Hypericaceae	Shrub
Helichrysum citrispinum	Asteraceae (Compositae)	Herb
Kniphofia foliosa	Asphodelaceae	Herb

Appendix 10. Insect body parts photographed with digital camera through the eye piece of a microscope during fecal sample analysis (Photo: Abebayehu Desalegn, 2015).

Appendix 11. Visiting cliffy areas in search of Ankober Serin at Mesareria.

Appendix 12. Mist netting at GFS II (A) and chenek (B)