

**HETEROPHIL: LYMPHOCYTE RATIO AS PHYSIOLOGICAL RESPONSE
INDICATOR TO FOREST FRAGMENTATION AND DEGRADATION IN
CABANIS'S GREENBUL IN TAITA HILLS**

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for conferment of a degree in any other University or for any other award.

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Declaration by the supervisors

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as University supervisors.

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DEDICATION

I dedicate this work to my family, especially my wife Juliet Mbaka Otieno for her support during my studies.

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LIST OF ABBREVIATIONS

1. **CFA** - Community Forest Association (CFA) is a community-based organization (CBO) that is formed by local community living near and depend on a forest, with the goal of managing and conserving the forest sustainably to improve their livelihoods
2. **F** – forest generalists – These are birds that is a bird species that can live and thrive both inside forests and in other habitats like farmlands, grasslands.
3. **f** – forest visitors – These are birds that use forests occasionally but do not depend on the forest as their main or exclusive habitat.
4. **FF** - forest specialist birds – these are birds that are strongly depended on forests for nesting, food, etc.
5. **FAO** - Food and Agriculture Organization of the United Nations.
6. **FCMA** - Forest Conservation and Management Act
7. **H:L** - Heterophil: Lymphocyte Ratio
8. **HPA** - Hypothalamic-pituitary-adrenal
9. **KFS** – Kenya Forest Service
10. **PFM** – Participatory Forest Management
11. **UNEP** – United Nations Environment Programme
12. **LiDAR** - Light Detection and Ranging

ABSTRACT

Habitat fragmentation and degradation are major stressors for tropical forest birds, yet their physiological consequences remain poorly understood. In response to stressors, birds and other vertebrates mount stress responses, which result in the release of the steroid hormone corticosterone into the bloodstream, which can then activate a behavioural and physiological response to the stressor. Corticosterone response is mounted within minutes of encounter with a stressor. It thus is regarded as better measure of short-term stressors like predator attack. In contrast, the Heterophil-to-Lymphocyte (H:L) ratio is a better indicator of long-term environmental stressors, such as habitat fragmentation and degradation. This study examined chronic stress in breeding females of Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*), a forest specialist species, across eight forest fragments (two larger – Ngangao and Chawia, and six smaller degraded – Susu, Ndiwenyi, Fururu, Iyale, Msidunyi, and Vuria), in Taita Hills, Kenya. The main objective was to determine how habitat fragmentation and habitat quality influence physiological stress levels during the breeding season. Birds were captured using mist nets, ringed, and blood samples collected for preparation of stained blood smears. Heterophil-to-lymphocyte (H:L) ratios were quantified by differential leukocyte counts, while habitat quality was characterized and assessed using LiDAR-derived measurements of canopy cover and fragment size. A total of 100 individuals were sampled across three breeding seasons (2016/2017, 2018/2019 and 2019/2020). Data were analyzed using linear mixed models (LMMs) in R, with log-transformed H:L ratio as the response variable and fragment size, canopy cover, sex, and year as predictors. Ring number was included as a random effect to account for repeated sampling. Results showed that H:L ratios were significantly higher in smaller degraded forest fragments with reduced canopy cover, suggesting increased stress in degraded habitats. Unexpectedly, high H:L ratios were also recorded in the large forest fragments, possibly as a result of high competition or predation pressure. These findings demonstrate that both habitats fragmentation and degradation impose chronic stress on forest specialist species and highlights the urgent need for conservation and management of Taita Hills forest habitats.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Human population growth and associated anthropogenic activities are major drivers of changes in land-use (Foley et al., 2005) resulting in habitat fragmentation and degradation that has been found to reduce species richness within communities, change species composition and abundance. Habitat fragmentation and degradation can negatively impact forest bird populations by altering resource availability, which in turn can affect population size, individual fitness, behavior, distribution, and evolutionary dynamics (*Homyack, 2010; Hinam & Clair, 2008, Matthysen & Dhondt, 1998*). For example, Habitat fragmentation caused by extensive habitat loss can constrain reproductive success in some species by reducing home range size, lowering provisioning rates, and increasing the energetic costs associated with breeding (*Hinam & Clair, 2008*), this can significantly contribute to pressure. The implications of prolonged pressure are widespread and greatly compromise an individual's well-being and ability to thrive which is influenced by various external and internal factors (*Lebigre et al., 2012*).

Long-lasting environmental changes caused by habitat fragmentation and degradation may challenge the ability of individuals to obtain sufficient resources from their environment and exacerbate trade-offs between reproduction and self-maintenance. Physiological responses related to energy balance, response to stressors or immune function may give indications whether individuals are coping well with environmental change or not (*Hing et al., 2016*). For example, the concentration of glucocorticoids circulating in the body is commonly used as an indicator of how well an individual is coping with challenges within

its environment (*Wingfield, 2015*). However, glucocorticoids are often assessed with a single blood sample, while environmental change is causing long-lasting challenges. Prolonged pressure can profoundly affect the immune function of birds, thereby increasing their susceptibility to diseases (*Skwarska, 2018*). Thus, Heterophil-to-Lymphocyte (H:L) ratio has been suggested as an alternative measurement of stress in birds that can reflect long-term environmental changes (*Müller et al., 2011*). Leukocytes, such as heterophils and lymphocytes, have been used to measure immune function and physiological stress response because they play a crucial role in the immune system of birds (*Masello et al., 2008*). Reduced wellbeing has been linked to increased heterophils (*Goessling, Kennedy, Mendonça, et al., 2015*), potentially contributing to changes in the leukocyte profile that result in an elevated H:L ratio (*Davis et al., 2008*). Heterophils are produced in large numbers when birds are under pressure or infection, (*Skwarska, 2018*). As a result, birds experiencing stress and allocating energy towards surviving challenging conditions tend to have an increased production of heterophils compared to lymphocytes (*Wilcoxon et al., 2015*). Therefore, changes in the leukocyte profile can be a valuable tool for assessing stress levels in birds and determining the impact of stress on their fitness (*Lebigre et al., 2012*). The H:L ratio is a reliable stress indicator in birds, offering a simple way to assess immune activity and overall health in both adults and juveniles. It also helps gauge avian responses to stressors of varying durations, including changes in environment, social stress, and increased energy demands during breeding (*Skwarska, 2018*).

Habitat loss and fragmentation cause detrimental effects to tropical birds (*Visco et al., 2015*), (*Olivier & Aarde, 2017*). This can cause prolonged pressure, affecting immune activity and altering hematological parameters (*Skwarska, 2018*), elevating H:L ratios in

tropical birds (*Bonier et al., 2009*). It can also affect breeding success in birds. A study in the Eastern Arc Mountains of Tanzania indicated that nest failure rates for Afrotropical birds were up to 197 times higher in fragmented forests compared to continuous forests (*Newmark & Stanley, 2011*).

Habitat fragmentation is a major threat to the health of tropical birds and more research is needed to understand the full extent of this threat (*Messina et al., 2022*). Physiological responses of tropical species to habitat change are much less studied than temperate zone species. More studies are needed to fill these gaps and understand how habitat fragmentation impacts the wellbeing of birds in tropical forests.

This study examined the Heterophil to Lymphocyte (H:L) ratio of Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*), in response to habitat fragmentation and degradation in the Taita Hills Forests during the breeding season when energy demand is high. The Cabanis's Greenbul (Placid) is a forest specialist (*Bennun et al., 1996*) and a cooperative breeding bird that receives support from non-breeding helpers (*Russell, 2007*). This breeding system is in approximately 9% of all bird species (*Kingma, 2017*). The main objective of this study was to assess the effect of prolonged environmental pressure on breeding females of Cabanis's Greenbul (Placid) in the fragmented forests of Taita Hills, Kenya, during the breeding season. It was hypothesized that habitat fragmentation and degradation will lead to an increase in the H:L ratio Cabanis's Greenbul (Placid) during breeding, because in previous studies it was found that female placid greenbuls occupying larger home ranges in degraded forests with reduced canopy cover and show higher glucocorticoid level (*Apfelbeck et al., 2024*) indicating more challenging conditions in areas with low canopy cover than in areas with more intact canopy cover. Therefore, it was

predicted that Cabanis's Greenbul (Placid) breeding in smaller or more degraded forest would elevate H:L ratios than breeding Cabanis's Greenbul (Placid) from larger, less degraded forest fragments. The study also assessed how factors such as forest fragment size, food availability and number of nestlings influence Cabanis's Greenbul (Placid) during breeding. The potential impact on the fitness and well-being of Cabanis's Greenbul (Placid) during breeding by determining the H:L ratio as a physiological indicator was investigated.

1.2 Statements of the problem

Habitat fragmentation and degradation are among the leading threats to biodiversity, particularly in forests. These environmental changes can have profound effects on wildlife health, yet physiological responses to such environmental changes remain poorly understood. In the cloud forests of the Kenyan Taita Hills, Cabanis's Greenbul (Placid) inhabits forest fragments of varying size and quality. However, it is unclear how differences in habitat structure and fragmentation influence physiological stress, as measured by the heterophil-to-lymphocyte (H:L) ratio. Addressing this knowledge gap is crucial for understanding how habitat changes impact avian health and for informing conservation strategies in fragmented tropical landscapes

1.3 Justification

The growing human population has led to habitat loss and habitat degradation. Through anthropogenic activities, critical habitats have been fragmented and degraded, leading to increased environmental pressures. The study was conducted in the Taita Hills, located within the Eastern Arc Mountains, which form part of one of the world's 35 recognised

biodiversity hotspots. This landscape is rich in endemic species but has been increasingly impacted by human activities, resulting in the fragmentation of forest habitats. This study was conducted on the breeding females of the placid Greenbul in the 7 forest fragments to improve our understanding of the effects of habitat fragmentation and degradation on forest specialist species, especially during breeding, and subsequently advocate for conservation and management of their habitats.

1.4 Study objectives

1.4.1 Main Objective

To investigate how habitat fragmentation and habitat quality influence physiological stress levels in Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*) during breeding.

1.4.2 Specific Objectives

1. To assess long-term stress levels in Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*) during the breeding season in the Taita Hills forest fragments using the heterophil-to-lymphocyte (H:L) ratio
2. To analyse how H:L ratios vary with forest fragment size and indicators of forest degradation (e.g., canopy cover and vertical vegetation structure).

1.5 Research questions

1. How does fragment size affect H:L ratio?
2. How does canopy cover affect H:L ratios?
3. Do birds in smaller degraded fragments show higher H:L ratios than those in larger, less degraded fragments?

1.6 Hypothesis

H₀ – Habitat fragmentation and degradation will not lead to an increase in the H:L ratio in Placid Greenbuls during breeding in smaller, more degraded forest fragments than in Cabanis's Greenbul (Placid) larger, less degraded forest

H_A – Habitat fragmentation and degradation will lead to an increase in the H:L ratio in Placid Greenbuls during breeding in smaller, more degraded forest fragments than in Cabanis's Greenbul (Placid) from larger, less degraded forest

1.7 Scope of the study

This study focuses on assessing the physiological responses of Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*) to environmental pressures due to forest fragmentation and degradation during breeding in the Taita Hills forests (eight cloud-forest fragments (two larger – Ngangao (120 ha), and Chawia (86 ha), and six smaller degraded ones – Susu (15 ha), Iyale (15.7 ha), Msidunyi, (20.9 ha), Vuria, (70.3 ha). Ndiwenyi (4 ha), Fururu (8 ha,) in the southeastern part of Kenya. The study measured habitat quality through assessing canopy cover, fragment size, and how these relate to individual bird stress levels, using the heterophil-to- lymphocyte (H:L) ratio as a physiological stress indicator. Data for this study were collected during three breeding seasons in 2016/2017, 2018/2019 and 2019/2020 with a total of 100 individual sampled (71 individuals (66 adult females and 5 adult males) from 2018/2019 and 2019/2020 and 29 individuals (18 adult females and 11 adult males) from 2016/2017.

1.8 Limitation of the Study

The study faced some limitations, including the difficulty in accessing a suitable microscope for differential counting. To overcome this, I received a mobility scholarship to Salzburg University, which enabled me to access a suitable microscope and perform the necessary analyses.

Although increased H:L ratios in larger fragments may suggest higher stress, the exact causes (e.g., predation pressure, competition) were not directly measured during this study, but this has been addressed and referred to prior studies in Taita Hills forest fragments showing higher nest predation in the largest forest fragments.

1.9 Assumption of the study

The assumption of this study is that habitat fragmentation and degradation can cause environmental pressures that negatively impact the physiological well-being of Cabanis's Greenbul (Placid) by increasing their H:L ratios. It is assumed that the H:L ratio is a reliable indicator of long-term environmental stress. I also assumed that forest fragment size and canopy cover are good proxies for habitat quality, and that reduced canopy cover and smaller fragment size represent more degraded conditions, while large fragments with a closed canopy represent a pristine habitat.

1.10 Definition of key terms

1. **Basophils** – They are a type of white blood cells that are dark-staining granules in the cytoplasm that obscure the nucleus
2. **Differential counting** - Measures the percentages of each type of White Blood Cell (neutrophils, eosinophils, and basophils, lymphocytes and monocytes) present in an individual bird

3. **Environmental stress** - a negative subjective psychological response to an environmental stimulus
4. **Environmental Pressure** - Natural and human-induced factors that bring about changes in the state of the environment and negatively impact the living organisms
5. **Eosinophil** – Is a white blood cell containing granules that are readily stained by eosin. It is one of the immune system components responsible for fighting multicellular parasites and certain infections in vertebrates.
6. **Glucocorticoids** - are a class of steroid hormones, naturally produced by the adrenal glands, and also available as synthetic medications
7. **Heterophil: lymphocyte (H: L) Ratio** – A metric used in avian and sometimes other animal studies to assess an individual's physiological stress response
8. **Habitat fragmentation** - A process during which a large expanse of habitat is transformed into a number of patches of a smaller total area, isolated from each other by a matrix of habitats unlike the original
9. **Habitat degradation** - A process where a natural habitat is made less suitable for the species that typically live there, without completely destroying the habitat
10. **Heterophil** - A type of white blood cell found in birds and some other non-mammalian vertebrates that are analogous to neutrophils in mammals. They are the primary phagocytic cells in these animals and play a crucial role in the innate immune response
11. **Leucocytes** – They are also known as white blood cells, are a crucial part of the immune system, defending the body against infection and disease.
12. **Lymphocytes** - A type of white blood cell that plays a crucial role in the immune system, helping the body fight off infections and diseases

13. **Monocyte** – They are part of Leucocytes that are first line of immune defense, acting as phagocytic cells that engulfs and destroys pathogens
14. **Staining** – This is a technique in microscopy and histology that uses dyes to increase contrast and visibility of biological specimens, often following a multi-step process including applying primary and counterstains, and sometimes a decolorizer and mordant.
15. **Cooperative breeding** – This is a breeding system in birds where several individual birds work together to help parents (breeding male and breeding female) care for offspring. The helpers, often relate, contribute to tasks like defending the nest and feeding the young, which increases the nest success rate and survival of the offspring
16. **PFM** - Participatory Forest Management (PFM) is a system of forest management that involves the collaboration of governments, communities, and other stakeholders in decision-making, resource utilization, and the sustainable management of forest resources

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter consists of reviews from secondary data that other researchers have collected from other journals, statistical reports, and primary data. Besides, it also outlines theoretical reviews regarding the variables to inform the researcher, conceptual framework and identify the study gaps. The study covers following areas; forest fragmentation and degradation, H:L ratio and Cabanis's Greenbul (Placid) in Taita Hills forests.

2.1 Forest fragmentation and degradation

Forest management in Kenya and their general status.

Kenya encompasses a diverse array of forest ecosystems, including montane and upper highland “water tower” forests such as the Mau Complex, Mount Kenya, and the Aberdare Range, as well as savannah woodlands, dry forests, coastal forests, mangroves, and dryland woodlands. These forest types are critical for biodiversity conservation and provide essential ecological and socio-economic functions, including water regulation, timber and energy supply, and carbon sequestration. Broadly, Kenya's forests are categorized into two main types: indigenous forests and plantation forests. (*Peltorinne, 2004; UNEP, 2020; Ototo & Vlosky, 2018*). As of 2021, forest cover in Kenya accounted for approximately 8.8% of the country's total land area, which remains below the 10% minimum target stipulated in the 2010 Constitution (*KIPPRA, 2023*).

Kenya's coastal forests, particularly the Arabuko-Sokoke Forest and the fragmented Taita Hills forests, are notable for their high levels of endemism. The Arabuko-Sokoke Forest

alone supports around 230 bird species, including several globally threatened taxa, such as the Sokoke Scops Owl and Clarke's Weaver. (Ngumbau *et al.*, 2020). The montane and rainforest ecosystems of the Mt. Kenya and Aberdare complexes, together with the Mau and Cherangani ranges, function as critical national "water towers," essential for regulating hydrological processes that sustain agriculture, hydropower production, and urban water supply. These forests are biodiversity hotspots and deliver a range of ecosystem services, encompassing provisioning services such as timber and fuelwood, regulating services including water flow and climate moderation, as well as important cultural and supporting functions (Osewe *et al.*, 2024). A study has showed that applying the triad forest management approach could enhance biodiversity conservation by integrating intensive timber zones, conservation areas, and multi-use (Chisika & Yeom, 2025).

Forests in Kenya are facing numerous threats, including agricultural expansion at forest margins, the collection of fuelwood and charcoal production, unclear boundaries and encroachment, and governance capacity gaps. These factors would lead to forest fragmentation and degradation.

Forest fragmentation and degradation have continued despite reforestation and conservation efforts. The degradation and deforestation are due to factors like agricultural expansion, charcoal production, illegal logging, and infrastructure development (UNEP, 2020;FAO, 2020). Wildfires threats have become frequent and intense, especially in dry seasons, intensified by climate change and human encroachment.(Rotich *et al.*, 2025) Forest management in Kenya has progressed significantly through policy reforms, community engagement, and collaborative initiatives. Traditionally, forest governance was highly centralized under colonial and early post-independence legislation. This centralized

approach was reformed with the enactment of the Forest Act of 2005, followed by the Forest Conservation and Management Act (FCMA) of 2016, which prioritized Participatory Forest Management (PFM) and strengthened community involvement in forest governance. (Chisika & Yeom, 2024) through formation of Community Forest Associations (CFAs) to support in managing the public forests in collaboration with Kenya Wildlife Service (KFS). This approach has been helped increase forest awareness like the Lembus Forest, where forest cover improved between 2000 and 2014 (*Kimutai & Watanabe, 2016*) However, the implementation of the PFM has faced challenges such as delays in formalizing management agreements, lack of capacity in CFAs, and inadequate benefit-sharing mechanisms(Osewe et al., 2025)

2.1.1 Factors causing forest fragmentation and degradation

Forest ecosystems in Kenya play a vital role by offering essential ecosystem services such as water catchment protection, climate regulation, and livelihood support. However, over the past few decades, these forests have increasingly suffered from fragmentation and degradation, endangering both biodiversity and the services they provide. Rising human populations have led to encroachment and illegal settlements within forest areas, largely driven by poverty and weak law enforcement. This issue is particularly evident in the Mau Forest, where encroachment has resulted in edge-dominated forest fragments with heightened levels of disturbance. (*Jebiwott et al., 2021*).

The following are some factors that are causing forest fragmentation and degradation in Kenya

Agricultural Land Expansion – The conversion of forested areas into agricultural land remains one of the primary causes of deforestation in Kenya. Forest ecosystems such as

those in the Aberdares and Mount Kenya regions have experienced significant clearance to accommodate both subsistence and commercial farming activities. (Peter Wass, 1995). Satellite imagery analyses indicate that substantial portions of the Eastern Mau Complex and Kakamega Forest have undergone extensive conversion to agricultural land (Were et al., 2013). A study on Kenya's forest-risk commodities revealed that smallholder shifting cultivation is the primary cause of forest fragmentation and degradation, contributing to roughly 85% of total forest loss, while commercial agriculture and mining add further pressure. (Kerstin Canby, Arthur Blundell, 2024).

Land Tenure Conflicts and Poor Governance – Studies have shown that when there is a lack of formal or secure rights to land, people are less likely to engage in sustainable forest management practices, as the risk of eviction or dispossession discourages long-term planning (Angelsen. et al., 2009). Forest lands are frequently allocated for private development in an unregulated manner, which compromises conservation and protection efforts (Qureshi et al., 2012). Illegal timber harvesting and charcoal production remain major causes of forest degradation, despite existing legal protections. Unauthorized logging persists even in gazetted forests such as Kakamega and the Taita Hills, leading to canopy gaps, a decline in species richness, and hindered regeneration of young trees (Kung'u et al., 2023). There are also overlapping institutional mandates between the Kenya Forest Service (KFS), County Governments, and the Ministry of Environment which reduces coordination.

Forest Fires and Natural Disturbance – Forest fires are a significant driver of forest fragmentation and degradation, particularly impacting plantation areas, natural forests, and montane regions during the dry season. These fires, which have become more frequent and

severe in recent years, are commonly linked to land-clearing activities. Studies have further identified agricultural burning, pasture management practices, and accidental ignitions as the primary sources of such fires (*Rotich et al., 2025*). A study has showed that for the period 1990–2024, wildfires destroyed over 50,000 ha of forest, shrubland, and grassland between 2003 and 2012, affecting ecosystem services, biodiversity, and soil health (*Rotich et al., 2025*).

2.1.2 Impacts of forest fragmentation on wildlife

The increasing human population has led to habitat fragmentation and degradation (*Siljander et al., 2009*). Forest biodiversity is threatened by habitat fragmentation and degradation, especially avian species. This caused long term pressure on birds to thrive in these habitats (*Bytebier, 2001*). Forest fragmentation and degradation alter the physical structure and ecological functioning of forest habitats, leading to the isolation of wildlife populations. (*Andren, 1999*) which would contribute to changes in species composition, behavioural shifts, and physiological stress in wildlife. A study in Kakamega indicates bat communities are influenced by factors such as fragment size, canopy density, and the extent of human disturbance. Smaller forest fragments and edge habitats typically harbor fewer insectivorous bat species, which are more commonly associated with forest interiors(*Webala et al., 2019*). The changes can affect birds as would be reduced food availability, increased risk of predation, and increased competition for resources (*Mazerolle & Hobson, 2002*). Bird nest predation rates are affected by structural changes and edge effects, which can sometimes lead to higher mortality in small passerines due to shifts in temperature conditions or predator activity (*Spanhove et al., 2009*).

Studies have shown that there is compelling evidence that fragmentation causes physiological stress in birds. Northern Saw-whet Owls (*Aegolius acadicus*), for example, reduced home range sizes and limited food availability in fragmented habitats have been linked to decreased reproductive success (Hinam & Clair, 2008). Similarly, (Suorsa, 2004) found that Eurasian tree creepers (*Certhia familiaris*) living in small forest fragments exhibited higher levels of stress hormones and reduced immune function compared to individuals in larger, continuous forest habitats

In Kenya, studies in the River Tana floodplains have showed that forest fragmentation adversely impacts primate populations. As forest patches became smaller and more isolated, primates experienced population declines associated with reduced habitat quality and limited food resources. (Wahungu et al., 2005). This was the same in another study on the Tana River mangabey (*Cercocebus galeritus*), which found that fragmentation affected both behavior and access to plant foods (Maingi, 2019).

Several studies have demonstrated that birds display behavioral adaptations in response to habitat fragmentation. For instance, cooperative breeding species modify their parental investment strategies depending on whether they inhabit fragmented or continuous landscapes, likely as an adjustment to changes in resource availability (Luck, 2017). Studies have shown that cooperative breeders display altered behaviors relative to non-cooperative species when living in fragmented habitats (Apfelbeck et al., 2024). In particular, individuals from cooperative groups exhibited lower stress hormone levels and more consistent social behaviors in fragmented habitats, indicating that group living may help mitigate environmental stress (Apfelbeck et al., 2024).

2.1.3 Approaches to conserve fragmented and degraded forests

Conservation of fragmented and degraded forests in Kenya has been largely dependent on community actions, particularly through mechanisms such as Community Forest Associations (CFAs). The Forest Conservation and Management Act (2016), has empowered CFAs to co-manage forests with the Kenya Forest Service (KFS) (*Kenya Forest Conservation and Management Act, 2016*). The Adopt-a-Forest model represents an innovative approach that utilizes public-private partnerships to rehabilitate degraded forest areas through commitments of three to five years. This initiative has contributed to the restoration of approximately 480,000 hectares of degraded forests, fostering active participation from corporations, non-governmental organizations, and individual stakeholders (*Chisika et al., 2024*). An agroforestry buffer strategy has been employed in fragmented regions such as the Taita Hills where 10-meter-wide agroforestry belts, with species like *Grevillea robusta* and *Albizia gummifera*, along forest edges mitigate edge effects and reduce pressure on core forest areas. These belts not only serve ecological functions but also support crop production and soil fertility, offering economic incentives to local communities (*Chisika et al., 2024*).

2.1.4 Forest biodiversity (birds)

Kenya is endowed with more than 1059 bird species (*BirdLife International, 2025*) this is attributed to the diversity of habitats, including forests. Forests play a crucial role in biodiversity conservation, serving as vital habitats for bird species. In Kenya, native forests provide refuge for approximately 299 bird species (*Mutuku, 2007*). Bird species that depend on forests are generally grouped into three categories based on how strongly they rely on forest habitats: forest specialists (FF), forest generalists (F), and forest visitors (f).

Forest specialists are typically found in intact, undisturbed forest interiors and are rarely encountered in degraded or secondary forests. Forest generalists are present within forest interiors and are more flexible using forest edges or altered habitats, although they still depend on forests for essential resources. Forest visitors mainly live in non-forest areas and only occasionally enter forested environments, without being dependent on them (*Bennun et al., 1996*)

The forest fragments in Taita Hills are rich in biodiversity and support endemic plant and animal species, including three bird species confined to these forests. They include Taita Thrush (*Turdus helleri*), Taita Apalis (*Apalis fuscigularis*), and Taita White-eye (*Zosterops silvanus*). These species are confined to small, isolated forest patches, making their populations particularly vulnerable due to habitat fragmentation and limited distribution. (*Brooks et al., 1998*) Cabanis's Greenbul (Placid) are forest specialists that rely on forest interiors for breeding, and a study has shown that forest degradation can influence their movement patterns and behavior. (*Kung'u et al., 2025*)

2.2 Heterophil-to-Lymphocyte (H: L) Ratio

Heterophils and lymphocytes, are very important in the immune system of birds (*Campbell, 2015*). In birds, physiological stress typically causes an increase in heterophils and a decrease in lymphocytes; therefore, the heterophil-to-lymphocyte (H:L) ratio is commonly used as an indicator of stress and immune function (*Goessling, Kennedy, Mendonc, et al., 2015*). Heterophils are phagocytic cells that play a key role in the innate immune response, with their numbers typically increasing in circulation during periods of infection, tissue injury, inflammation, or exposure to environmental and physiological stressors. In contrast, lymphocytes are central components of the adaptive immune system, mediating responses

to specific pathogens such as viruses and parasites. Elevated heterophil counts, often resulting from stress, compromised health, or corticosterone administration, can alter leukocyte distributions and consequently increase the heterophil-to-lymphocyte (H:L) ratio (*Davis et al., 2008*). Therefore, changes in the leukocyte profile can be a valuable tool for assessing the impact of environmental change on birds.

Studies have showed that the H:L ratio is higher in offspring or adults breeding in low quality habitat (*Johnstone et al., 2012, Maron et al., 2012*). For example, in forests at northern latitudes forest management practices or forest patch size are related to H:L ratios in breeding birds and offspring (*Suorsa, 2004*) and are predictive of nestling mortality. Habitat loss and fragmentation cause long-term detrimental effects in tropical forest birds (*Visco et al., 2015*). Meanwhile more research is needed to understand the full extent of physiological responses of tropical species to habitat change (*Messina et al., 2022*). While most studies have focused on effects of habitat change on temperate birds, their results may not be fully applicable to tropical birds because of their different life history and their evolutionary history in less variable environments (*Wiersma et al., 2007*). More studies are needed to better understand how habitat fragmentation and degradation affect the health and well-being of birds in tropical forests. The heterophil-to-lymphocyte (H:L) ratio is widely regarded as a robust indicator of chronic or long-term stress in avian species, particularly in response to environmental disturbances such as habitat fragmentation and degradation. In contrast, corticosterone (CORT) concentrations typically reflect short-term or acute stress responses due to their rapid fluctuation. (*Davis et al., 2008*). Corticosterone (CORT) concentrations can increase within minutes following capture, potentially confounding hormonal assessments. In contrast, the heterophil-to-lymphocyte (H:L) ratio

exhibits a slower response to stress, making it less susceptible to short-term disturbances such as handling. Owing to this delayed physiological adjustment, the H:L ratio serves as a reliable indicator of prolonged stressors, including food limitation, high temperatures, overcrowding, and disease.(*Davis et al., 2008*). H:L ratios and CORT do not always correlate, this may indicate that they may reflect different aspects of stress response (*Müller et al., 2011*). Birds in degraded habitats usually have high H:L ratio indicating chronic stress likely due to limited resources and increased parasite exposure (*Carbó-Ramírez & Zuria, 2015*) Birds in fragmented landscapes have their health affected (*Shirima & Werema, 2017*), (*Mbegu, 2011*). Wilcoxon et al indicated that even beneficial human interventions, such as bird feeding, can result in higher H:L ratios due to increased social and pathogen stress(*Wilcoxon et al., 2015*).

2.3 Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*) in Tata Hills

The Cabanis's Greenbul (Placid) (*Phyllastrephus cabanisi placidus*) is a subspecies of Cabanis's Greenbul (*Phyllastrephus cabanisi*) is a songbird belonging to the Pycnonotidae family, it is an insect-eating forest specialist that breeds in pairs or small family groups within the cloud forests of Eastern Africa (*Bennun et al., 1996*). The Placid Greenbul occurs in eastern Kenya, where it mainly occupies dense undergrowth and the mid-canopy layers of forests. Its preferred habitats include subtropical or tropical dry forests, moist lowland forests, and moist montane forests. In Kenya, it is restricted to the fragmented cloud forests of the Taita Hills in the southeast. The species is characterized by olive-green plumage, a bright yellow belly, and a distinctive white throat patch.



Figure 1. Female Phyllastrephus cabanisi placidus incubating © Beate Apfelbeck

Placid Greenbuls exhibit cooperative breeding behavior, which appears to be an adaptive strategy in fragmented habitats where factors such as increased nest predation and limited territory availability make independent breeding more challenging (Loock *et al.*, 2017). Helpers contribute by feeding nestlings, defending the territory, and possibly enhancing the survival of fledglings after they leave the nest. Their assistance generally benefits the breeding pair, though the effectiveness of cooperation can vary depending on factors such as helper performance, social structure, territory quality, and relatedness among group members (Winckel, 2014). Environmental pressures and the size of forest fragments influence both reproductive investment and helping behavior. (Loock, 2019). Other studies

showed in other cooperative breeders that ecological uncertainty and helper presence can influence maternal investment, such as egg size and clutch number (*Russell et al., 2007; Langmore et al., 2016*). The trade-off between the benefits and costs of cooperative breeding in the Placid Greenbul represents a flexible strategy to cope with environmental fluctuations and reproductive uncertainty. In the Taita Hills, studies on forest fragments and local populations have examined territoriality, breeding patterns, and the species' sensitivity in movement and foraging to habitat disturbance and fragment size, revealing behavioral traits that increase their vulnerability to fragmentation (*Apfelbeck et al., 2024*)

2.4 Conceptual Framework

Figure 2 represents the conceptual framework of this study, which links habitat fragmentation and degradation to chronic stress in the forest specialist, Placid Greenbul. It indicates that reduced habitat quality measured through factors like canopy cover and fragment size will lead to long-term environmental stress, which can be detected through elevated heterophil: lymphocyte (H:L) ratios in Placid Greenbul. The framework illustrates how habitat changes intensify ecological pressures, such as competition and predation, which in turn influence stress levels.

At the centre of this study is the relationship between habitat quality and the physiological response of the Placid Greenbul to environmental pressure. The habitat quality was measured through variables such as canopy cover, fragment size, and vegetation structure. Long-term environmental pressure in birds can be detected by examining the H:L ratio in blood. By linking ecological variables to physiological indicators, the framework provides

an understanding of how habitat changes at the Taita Hills fragmented forests translate into measurable impacts on Placid Greenbul.

Forest specialists such as the Placid Greenbul depend on forests; however, fragmentation and degradation pose multiple challenges. The loss of canopy cover also exposes birds to harsher conditions, including higher predation risk and increased energetic demands for thermoregulation. In this way, both fragmentation and degradation directly reduce habitat suitability for the Placid Greenbul environments that impose higher ecological pressures.

The reduction in habitat quality due to fragmentation and degradation indirectly influences stress levels in birds by intensifying ecological pressures. As suitable habitat patches become smaller, bird populations are forced into tighter spaces where they must compete for limited food resources, nesting sites, and territories. For a forest specialist like the Placid Greenbul, which depends on vegetation structures for foraging and breeding, increased competition with both specialist and generalist species can result in greater energetic costs and social stress, especially during the breeding season. Predation risk is another ecological pressure heightened by habitat change. Habitat changes may alter the abundance and distribution of insects, which are important food resources for the Placid Greenbul. Reduced food availability will force individuals to use more energy searching for food, leading to nutritional stress. Predation and food scarcity represent interconnected ecological stressors that arise directly from changes in habitat structure and quality.

The ecological pressures associated with habitat change ultimately manifest in the physiological condition of birds. Chronic stress is an adaptive physiological process in which the hypothalamic-pituitary-adrenal (HPA) axis is activated to help an organism cope with prolonged environmental challenges. While acute stress responses may be beneficial

in the short term, chronic activation of stress pathways has negative consequences for immune function, growth, and reproduction. In birds, one of the most reliable long-term indicators of chronic stress is the H:L ratio. Unlike measures of circulating glucocorticoid hormones, which can fluctuate within minutes due to handling or acute disturbances, H:L ratios provide a more stable measure of chronic stress exposure.

By examining H:L ratios in Placid Greenbuls across habitats that vary in canopy cover, fragment size, and degree of degradation, this study can directly link ecological changes to physiological outcomes. Birds in smaller, more degraded fragments are expected to show higher H:L ratios, indicating greater levels of chronic stress compared to individuals in larger, intact forests.

This conceptual framework, therefore, integrates ecological and physiological perspectives to explain the consequences of habitat change for forest specialists. Habitat fragmentation and degradation represent the primary drivers, which reduce habitat quality through loss of canopy cover and reduced fragment size. The effect of long-term pressures is expressed physiologically as chronic stress, which is measured through high H:L ratio.

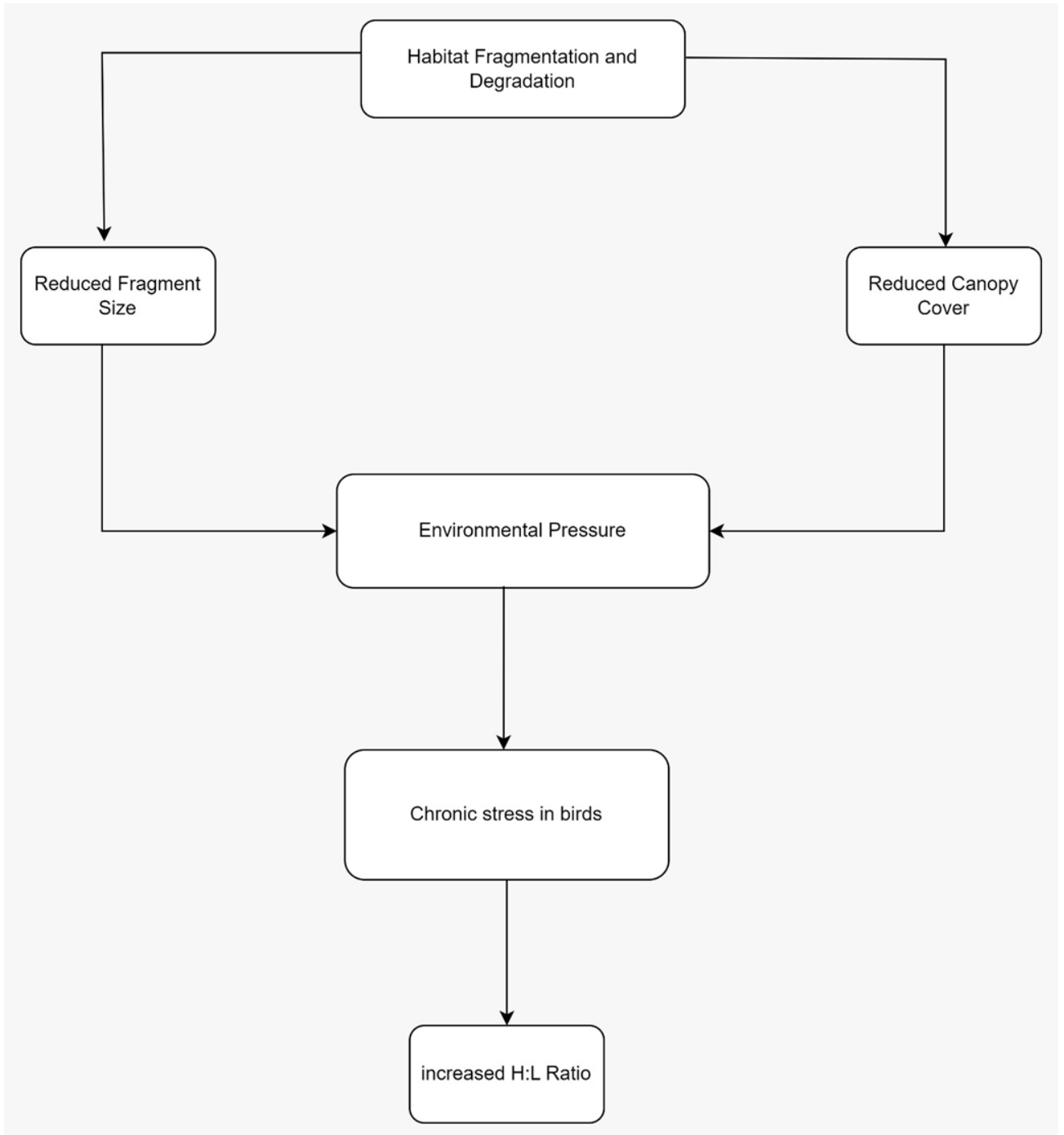


Fig 2. Conceptual framework of the study © Vincent Otieno Onyango

2.5 Summary

Habitat fragmentation alters ecological structures, resulting in reduced food availability, increased predation, and increased competition, which contributes to long-term stress in birds. Studies have shown that birds in fragmented and degraded habitats exhibit higher stress levels, as indicated by various biomarkers. However, there is a significant research gap in understanding how these stress responses are exhibited in tropical forest specialists, including the Taita Hills Forest Fragments, which contain confined endemic species. The focus of this study was on Placid Greenbul (*Phyllastrephus placidus*), a cooperative breeder. Studies have shown that the H:L ratio provides a more stable indicator of chronic stress. Chronic stress varies with habitat quality, and the social structure in this species remains poorly understood. In the next chapter (Chapter three), the research methodology employed to assess habitat quality, blood samples collection and analysis and quantify H:L ratios is outlined.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Introduction

This chapter elaborates on the study area, materials and methods employed in conducting this study. This study was conducted in Taita Hills forest fragments using an integrated research design that combined field blood sampling, laboratory analysis, and remote sensing techniques to investigate chronic physiological stress in breeding Placid Greenbuls. In total, 100 individual birds were sampled.

3.2 Study Area

Taita Hills

Taita Hills is in Southeast of Kenya, in Taita-Taveta County (3°25'S, 38°20'E). The mean annual rainfall is over 1500 mm in the upper mountain zones (*Chiew & McMahon, 2002*). The forests in Taita Hills are fragmented evergreen cloud-forest remnants that supports many endemic plants and animals, these forest fragments are home to critically endangered birds like the Taita Thrush and Taita Apalis, as well as rare butterflies and other invertebrates making them a recognized global biodiversity hotspot despite their limited size. The Hills form the northernmost part of the Eastern Arc Mountains (*Stam et al., 2017*) and is one of 35 global biodiversity hotspots (*Myers et al., 2000*). The Taita Hills are divided into three main blocks. Sagalla Hill (peak 1,450 m), directly south of Voi, is separated from the rest of the hills by the Voi River on the plains. The main body of the hills, Dabida, lies 25 km north-west of Voi, including the high peaks of Ngangao (2,149 m) and Vuria (2,228 m). To the north-east of this range lies the massif of Mbololo (2,209 m), separated from the main block by a valley at c.900 m. Some 50 km to the south-east, and

not included within this IBA, lies Mount Kasigau. The forest on this isolated peak has biogeographical affinities with the Taita Hills, but its fauna and flora are little studied. Geologically, the hills are the northernmost outpost of the ancient Eastern Arc mountains of Tanzania and Malawi. At the base of the hills, rainfall is only c.600 mm/year. This rises to c.1,300 mm on the top, with local variation. Dry bushland runs up the flanks of the hills, giving way rather abruptly near the top to smallholder cultivation and remnant patches of moist forest. The area is heavily settled. The total population is around 250,000, and densities reach 1,400 people/km² in places. Cultivation is intensive, with maize the most conspicuous crop. As a result of the high human pressure on land, forest remains only as scattered fragments on the hilltops and ridges. The study was conducted in eight cloud-forest fragments (two larger – Ngangao (120 ha), and Chawia (86 ha), and six smaller degraded ones – Susu (15 ha), Iyale (15.7 ha), Msidunyi, (20.9 ha), Vuria, (70.3 ha). Ndiwenyi (4 ha), Fururu (8 ha,) (*Husemann et al., 2015*). The forest fragmentation and degradation in Taita hills forests threatens biodiversity in the forests especially the endemic species in these forests are increasingly confined to small, isolated patches (*Bytebier, 2001*) and (*Beentje et al., 2011*). Using remote sensing, Wekesa et al and Siljander et al indicated that the massive forest cover loss from 1955 to 2016 (*Wekesa et al., 2020*) and (*Siljander et al., 2009*) . This fragmentation has led to reduced connectivity, with significant impacts on birds, including changes in avian diversity and distribution (*Githiru et al., 2009*). Bird populations in fragmented habitats exhibit increased fluctuating asymmetry, a sign of developmental stress (*Lens et al., 1999*). Loock (2019) indicated that life-history traits of some species in the Taita Hills, such as cooperative breeding, may be shaped by fragmentation (*Loock, 2019*).

The study was conducted in eight cloud-forest fragments (two larger – Ngangao (120 ha), and Chawia (86 ha), and six smaller degraded ones – Susu (15 ha), Iyale (15.7 ha), Msidunyi, (20.9 ha), Vuria, (70.3 ha). Ndiwenyi (4 ha), Fururu (8 ha,) (Husemann et al., 2015).

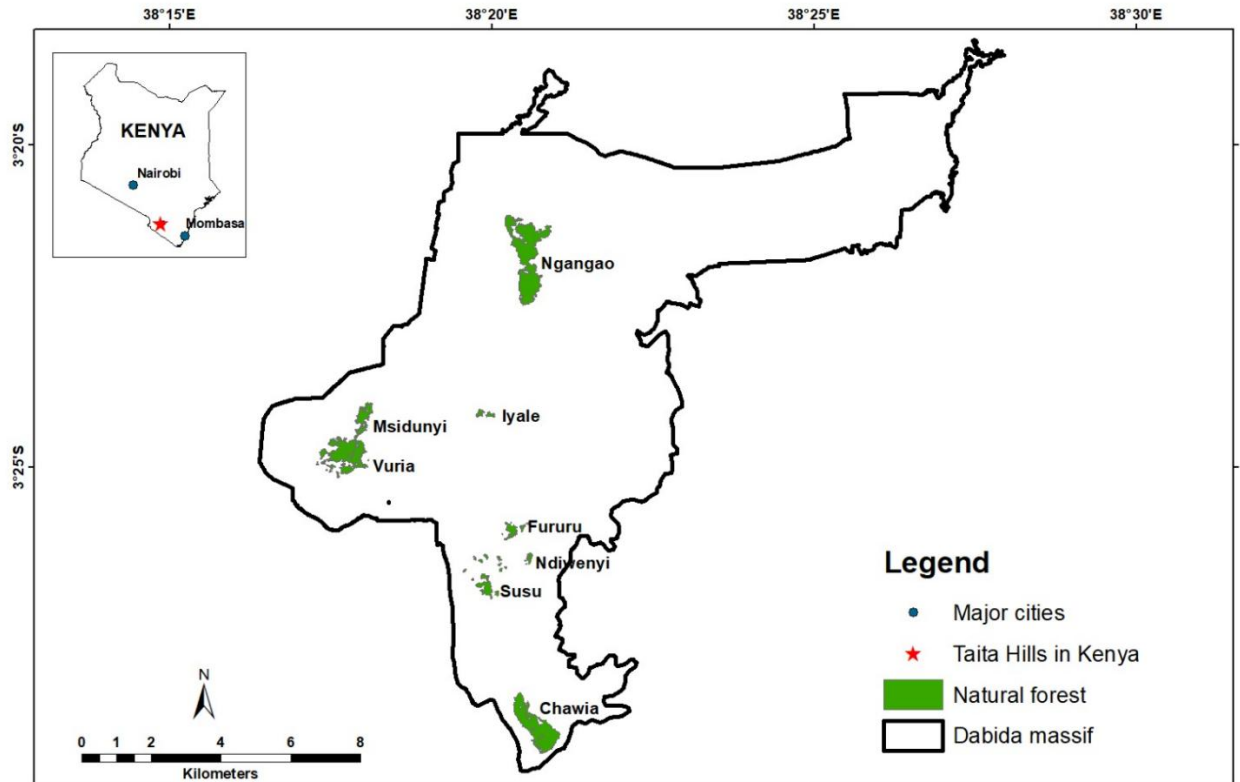


Fig. 3. Forest fragments under study in the Taita Hills, Kenya, are indicated in green. ©

Gladys Kung'u

3.1 Research design

This study employed an integrated research design that combined field-based sampling with laboratory analyses and remote sensing derived habitat assessments in order to evaluate the relationship between habitat quality and chronic physiological stress in

breeding populations of the Placid Greenbul. The study was conducted in Taita Hills forest Fragments. The study area provided an environment for examining the physiological impacts of habitat fragmentation and degradation.

H:L ratio was used as a physiological indicator as is a widely used and reliable measure of chronic stress in birds. Unlike plasma corticosterone assays, which reflect acute stress responses that can fluctuate rapidly in response to short-term disturbances, the H:L ratio provides a more integrated index of stress exposure over longer time scales. This makes it particularly suitable for assessing the effects of chronic habitat degradation. Blood samples were collected in the field and later analyzed in the laboratory using standard hematological techniques to determine the relative proportions of heterophils and lymphocytes.

Fieldwork was conducted during the breeding season, when birds are most likely to face heightened energetic demands and when the effects of habitat quality on physiological condition are expected to be most pronounced. Eight forest fragments in the Taita Hills were selected for study. These included both large forest fragments, generally assumed to be relatively pristine due to their size and small fragments, which were assumed to be more degraded owing to their high exposure to anthropogenic disturbance, greater edge effects, and altered microclimatic conditions.

Within each fragment, mist nets were employed to capture adult breeding Placid Greenbuls. Birds were carefully removed from the nets and handled in accordance with ethical ornithological guidelines to minimise stress during sampling. Immediately after capture, blood samples were taken from the brachial vein using sterile capillary tubes. To avoid artificially elevating leukocyte counts due to handling stress, sampling was conducted as quickly as possible, typically within three minutes of capture. Each individual bird that was

captured was then measured for basic morphometric traits, banded with an aluminum ring with a unique number and colour rings. The colour rings were well coded to help identify a particular individual without capturing again.

Blood smears were prepared in the field and later stained with a standard hematological stain (Giemsa, Wright's stain). Slides were examined under a light microscope at high magnification, and leukocytes were differentially counted by categorizing each cell as either a heterophil, lymphocyte, monocyte, eosinophil, or basophil. At least 100 leukocytes were counted per slide to ensure statistical reliability. The H:L ratio was calculated by dividing the number of heterophils by the number of lymphocytes, providing a quantitative index of each bird's chronic stress level. To minimize observer bias, counts were conducted blind to fragment identity, and a subset of slides was re-counted to ensure repeatability.

Physiological data were integrated with habitat quality assessments at the landscape scale. Remote-sensing data, particularly Light Detection and Ranging (LiDAR), were employed to generate detailed three-dimensional structural measurements of forest canopies across the forest fragments. LiDAR-derived variables included canopy height, canopy closure, and vertical complexity, all of which are indicators of habitat quality and correlate with forest degradation levels. These measurements were complemented by ground-based vegetation surveys, which involved recording tree species composition, basal area, and canopy cover using standardized forest inventory methods. Together, these data allowed for a robust characterization of habitat quality, ranging from structurally intact, floristically diverse large fragments to structurally simplified, species-poor small fragments.

The study design thus enabled direct comparison of physiological stress levels in birds across a gradient of habitat quality. By combining blood-based stress indicators with both

remote-sensing and ground-based measurements of forest structure, the research was able to test whether individuals inhabiting small, degraded fragments exhibited elevated H:L ratios relative to those in larger, more pristine habitats. Additionally, the use of multiple fragments per habitat category strengthened the generality of the findings, reducing the likelihood that patterns were driven by conditions in a single site.

3.3 Target population

The targeted population was Placid Greenbul populations in the eight forest fragments of Taita Hills. There were 100 individual birds sampled, this included 29 birds (18 adult females and 11 adult males) sampled in 2016/2017 and 71 birds (66 adult females and 5 adult males) sampled in 2018/2019 and 2019/2020. Blood samples were collected from these individual birds and thin blood smears were made for further microscopic blood analysis to obtain the H:L ratio.

3.4 Research and Sampling design

Species capture and processing

The study was conducted during the breeding season (November to March 2016 to 2017 and 2018 to 2020). In eight forest fragments greenbul nests were monitored and individuals were captured in the vicinity of nests using mist nets (3 m by 5 m). Upon capture, a blood sample was immediately taken within the first three minutes of capture or before release after processing the individual within 15 minutes. The individual's sex was determined by examining the presence of a brood patch (breeding females) and the size of the cloacal protuberance, which is larger in breeding males than in females. Subsequently, the bird was fitted with a unique combination of three-colour rings and a metal ring which made it easy

to identify and track individual birds without needing to recapture them. Other biometric measurements recorded included weight, wing length, and tarsus.

3.5 Data collection methods

Blood samples were obtained by puncturing the brachial wing vein and collecting blood within heparinized capillary tubes. A single-use syringe needles (5/8", or 1.6 cm length; 25 – 27 gauge) was used to extract blood from the brachial vein of the individual Placid Greenbuls. Heparinized (red; 50 - 75 µl) Capillary tubes were used to draw blood and put a drop on a clean slide to make a thin blood smear.

Later from the forest, slides were stained with a Wright-Giemsa stain following the manufacturer's instructions (Diff-Quick, LABOR + TECHNIK Eberhard Lehmann GmbH, Germany)(Stain et al., 2019).

Slides were stored in slide-boxes in a dry and cool area.

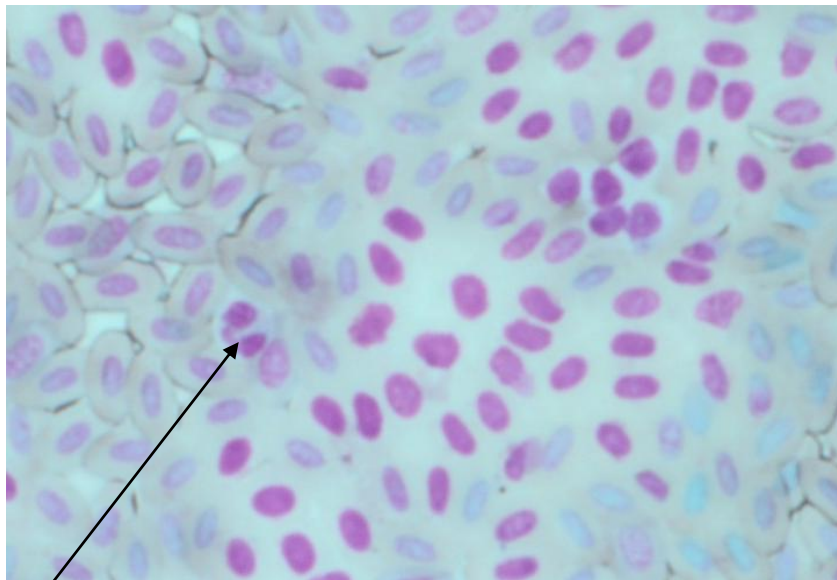
White Blood Cells in Birds

White blood cells are essential components of the avian immune system as serve as the body's defenders against invading pathogens such as bacteria, viruses, fungi, and parasites. There are types of white blood cells in birds, namely heterophils, lymphocytes, monocytes, eosinophils, and basophils. Each of these white blood cells plays a unique and important role in protecting and maintaining overall health of the bird.

The following are the five White Blood cells and their functions in Placid Greenbul

1. **Heterophils** are the most abundant granulocytes in birds, typically comprising between 40 and 75% of circulating white blood cells. They have a bilobed or trilobed nucleus that stain pinks and the cytoplasm is light pink or colorless. Heterophils are bird's first line of defense

during infection and are especially important in innate immunity. They attack invading microbes by engulfing and destroying them through phagocytosis. They play a crucial role in the avian immune system, with large numbers available to engulf and destroy bacteria and other pathogens. They are particularly active during acute or severe infections, inflammation, and in response to stress or low-grade infections. During infection, heterophils also release chemical mediators that trigger inflammation and they attract and activate additional immune cells at the site of tissue injury. When birds face bacterial infections or acute inflammation, heterophils increase rapidly in number, reflecting their importance in immediate defense. They increase in circulation during stress as part of the innate immune response. There were immature heterophils that their nucleus was not very clear but stain pink. The nucleus stains slightly darker than Cytoplasm for immature ones.



Photos of Heterophiles: Placid Greenbul ring no. AA19606 © Vincent Otieno Onyango
Heterophil

2. **Lymphocytes** – They are second most abundant white blood cell population in birds and are central to adaptive immunity. They are characterized by a single nucleus and the absence of cytoplasmic granules. They play a central role in adaptive immunity and typically decline in response to chronic stress. The lymphocyte has one big nucleus that stains pink to purple and a small cytoplasm that stains light blue

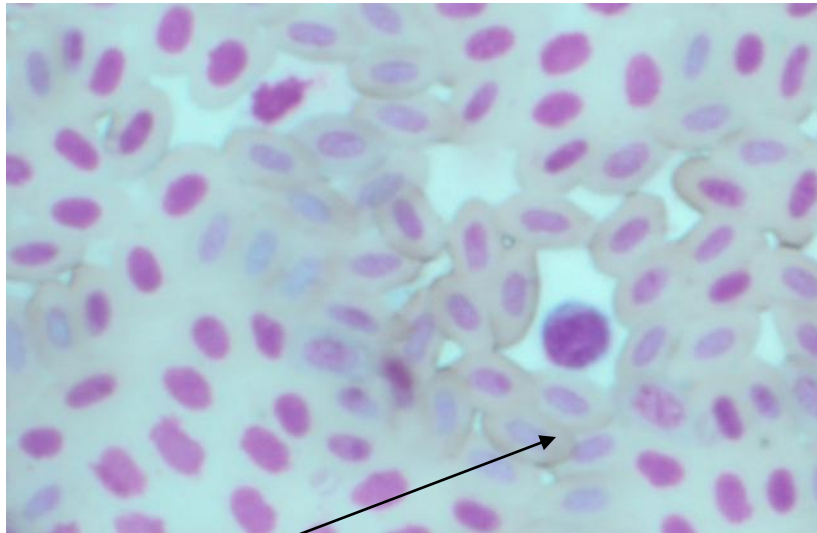


Photo of Lymphocyte: Placid Greenbul ring no. AA19606© Vincent Otieno Onyango
Lymphocyte

3. **Monocytes** are known as mononuclear WBC. They are large in size and have a shoe-like or bean-shaped nucleus that stains pink and they have a small cytoplasm that stains light blue. Monocytes resemble lymphocytes in appearance but are present in low numbers, typically ranging from 0 to 3% of circulating white blood cells (Gram, 2007). They are first line of immune defense, acting as phagocytic cells that engulfs and destroy pathogens

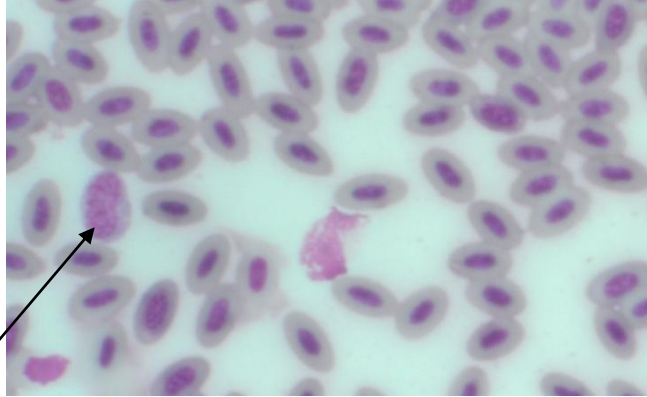


Photo of Monocyte: ring no. AA38823 © Vincent Otieno Onyango

Monocyte

4. **Basophils** are a type of white blood cell, specifically a type of granulocyte, that plays a role in the immune system. Basophil has a round to oval, nonlobed nucleus which usually obscured dark pink to purple granules

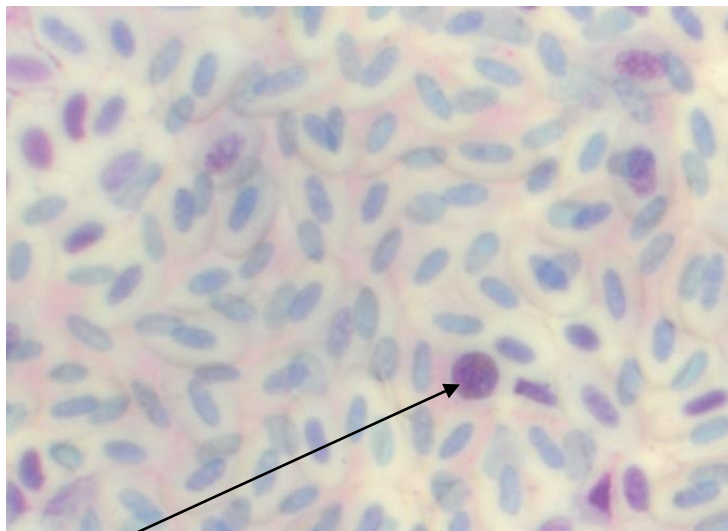


Photo of basophil: Placid Greenbul ring no. AA33099 © Vincent Otieno Onyango

Basophil

5. **Eosinophils** are granulated and they have a bilobed nucleus that stains pink. The cytoplasm stains blue and has granules. The eosinophils are not as many as heterophils, and the granules of the eosinophil will stain differently than those of the heterophil

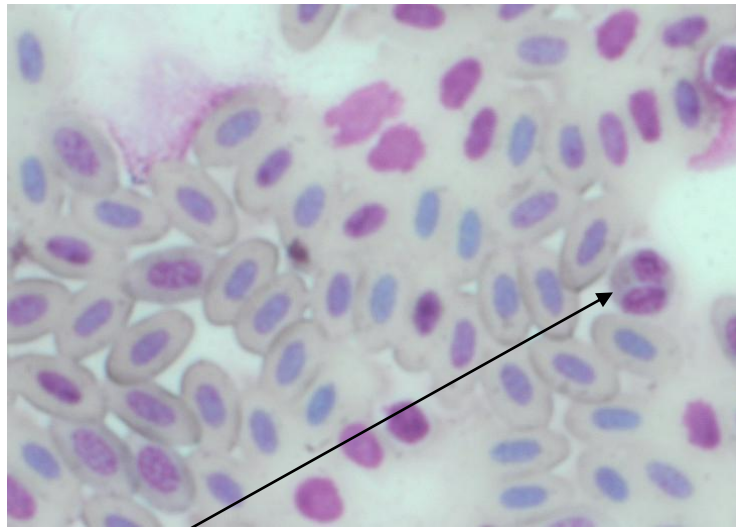


Photo of Eosinophil: ring no. AA38823 © Vincent Otieno Onyango

Eosinophil

White Blood Cell	Normal range %
Eosinophils	0 – 2
Heterophils	40 – 75
Monocytes	0 – 3
Lymphocytes	20 – 50
Basophils	0 – 5

Each cell type was counted, and to calculate the H:L ratio, the number of heterophils was divided by the number of lymphocytes. Samples of the two breeding seasons were analysed by two different people. To ascertain similarity between counts, the second person analysing slides from the breeding season 2019/2020 trained on samples from 2018/2019, and then analysed slides from the breeding season 2019/2020 until the values matched those of the previous counts. Training involved eight slides; high similarity between counts was reached after the fourth slide ($r = 0,99$). In addition, four samples from 2019/2020 were also analyzed twice ($r = 0,91$).

Differential counting of Heterophil to Lymphocyte Ratio

Differential counting of white blood cells was performed through examination of the stained thin blood smears using a compound microscope (Polyvar in Mikroskopierraum E-2.014) widefield photomicroscope where (10X) eyepiece lens and oil immersion objective (100 x) are necessary to attain 1000X magnification for 71 individuals (66 adult females and 5 adult males) from 2018/2019 and 2019/2020 in Salzburg University, Austria. 29 individuals (18 adult females and 11 adult males) from 2016/2017 were examined under a light microscope at the National Museums of Kenya at a magnification of x1000 (Dunn et al., 2013). The examination started with the lowest magnification objective lens. As the blood cells came into clear view, the higher magnification lenses were successively utilized until the oil immersion objective (x100) was reached. The examination started in an area where the cells were well distributed, typically near the tail end of the blood smear. Counting was then conducted in a snake-like manner (ECLinPath, 2025).

The examination started where the cells are well distributed/spread. This area is usually located near the wedge-shaped ends of the blood smear also called the tail. Proceed counting in a snake-like direction as shown below.

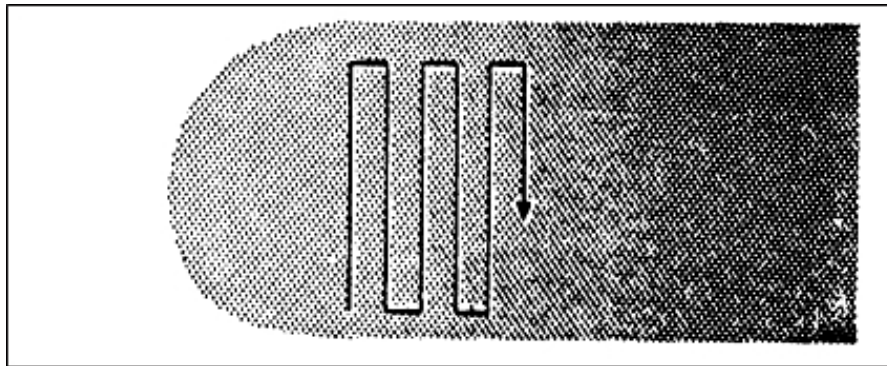


Fig 5 differential counting

During the examination, the morphology of white blood cells was assessed, and a differential count was performed. A total of 100 white blood cells per slide was counted, and the numbers of different cell types were recorded. Leukocytes were differentiated into heterophils, monocytes, lymphocytes, eosinophils, and basophils based on their characteristics, including shape, size, and staining properties of the nucleus, as well as the presence of granules (Campbell, 2015). For field view, it is better to explore the slide through the microscope eyepiece rather than the computer display, as the computer display has a smaller field of view.

3.6 Data analysis and presentation

Statistical analyses were conducted using R version 4.1.2 including the packages plyr (Wickham, 2011), lme4 (Bates et al., 2015) for linear models, and ggplot2 (Wickham, 2016)

for graphics. Model fit was confirmed by visually assessing normality and homoscedasticity of residuals.

3.7 Measurement of variables

Environmental variables

Forest degradation affects the stratification and structure of vegetation. This was therefore quantified vertical vegetation structure and canopy cover at the territory level. Vertical vegetation structure around nest sites was assessed in four subplots of 15 m radius per territory, i.e., a central subplot at the nest location and three subplots 50 m away from the nest location (50 m south, 50 m north-east, 50 m north-west) in April and May 2021 and 2022. In each subplot, the presence of vegetation was assessed by looking upwards. In each subplot, the presence of vertical vegetation structure (0/1) was recorded by looking upwards and assessing the presence or absence of vegetation (0/1) within a circle of 0.5 m radius at five points within a circle of 0.5 m radius in five height intervals (0-1 m, 1-5 m, 5-9 m, 9-15 m, >15) at five points. Vertical vegetation heterogeneity as vegetation layer diversity was calculated by calculating the Shannon-Wiener diversity index over the five vegetation height intervals, and as a percentage of canopy cover by summing all occurrences of vegetation above 9 m for the 20 sampling points per plot (*Bibby et al. 2012*). As vegetation layer diversity and canopy cover index were highly correlated ($r(143) = 0.8$, $p < 0.001$), we retained only the canopy cover index for further analysis as it showed higher variation. The canopy cover index was used as an estimate of the vertical vegetation structure within each forest and refer to it as such in the text.

The percentage of indigenous forest canopy cover at each nest site was calculated within a circle of 0.79 ha using light detection and ranging (LiDAR). LiDAR data were acquired at

a mean flight height of 1450 m above ground level using an aircraft-mounted Leica ALS60 sensor in January–February 2014 and February 2015 (*Adhikari et al., 2017*). LiDAR points were classified into ground points and non-ground points, and a digital terrain model (DTM) was computed at 1 m resolution using LAStools software (rapidlasso GmbH). A 3 m height limit was applied to separate ground/understory and canopy returns (*Adhikari et al., 2017*). Canopy cover was defined as a ratio of the first returns from canopy and all first returns (*Heiskanen et al., 2015*) and calculated using lidR package (*Roussel et al., 2020*) in R environment (*RCoreteam, 2023*).

3.8 Data Analysis Methods

R version 4.1.2 including the packages plyr (*Wickham, 2011*), dplyr for data manipulation, lme4 for linear models (Bates et al., 2015), and ggplot2 for graphics (*Wickham, 2016*) were used to conduct the data analysis.

The relationship between log-transformed H:L ratio and vertical vegetation structure, canopy cover and fragment size in breeding adult greenbuls using linear mixed models was tested. In addition to proxies for territory quality, sampling year, and sex were included. Body mass was not included in the analysis as body mass was largely explained by sex and was not significant when included in the model. Ring number was included as a random effect to account for individuals that had been sampled repeatedly in different years.

1.9 Empirical model and hypothesis testing

A linear mixed effects model was used where H:L ratio was the dependent variable and predictor variables included fragment size, canopy cover, vertical vegetation structure, sex, year.

The hypothesis that birds in larger forest fragments and areas with higher canopy cover would show lower H:L ratios was tested, showing reduced stress.

CHAPTER FOUR

RESULTS AND FINDINGS

4.0 Introduction

This chapter presents the results of the study highlighting the findings and significant outcomes that emerged from this study showing data analysis, presentation and interpretation. Discussions of the findings of this study are expanded in this chapter and also provide actionable recommendations that can guide future research, inform policy decisions, and conservation of the fragmented Taita Hills forests.

4.1 Data Analysis, Presentation and Interpretation

Greenbuls had higher H:L ratios in larger fragments than in smaller fragments as shown in the following table

Table 1. Descriptive statistics for the variation in H:L ratios between fragments. Fragments smaller than 21 ha are combined. Additionally, the analysis includes two samples from fragment Vuria, which is intermediate in size but mostly made up of exotic forest, with native forest occupying only a small area.

Fragment ID	mean	standard deviation	min	max	sample size
Ngangao	1.06	0.67	0.20	2.92	57
Chawia	0.79	0.50	0.17	2.15	25
Small fragments	1.01	0.94	0.25	3.5	15

Table 2. Statistics and coefficients for linear mixed-effects models determining the relationship between variation in H:L ratio of greenbuls nesting in areas of varying quality in cloud forest fragments of the Kenyan Taita Hills (97 observations of 81 greenbuls). CI, 95% confidence interval. Significant relationships are indicated in bold.

Predictors	Estimates	CI	df	t-value	p-value
Intercept [2016/17; female]	0.19	-0.09 – 0.48	87.5	1.36	0.18
Year [2018/19]	-0.38	-0.72 – -0.04	64.2	-2.21	0.03
Year [2019/20]	-0.60	-0.93 – -0.27	80.5	-3.60	0.0005
Residual body mass	-0.02	-0.11 – 0.08	84.6	-0.38	0.71
Sex [male]	-0.54	-0.93 – -0.14	84.0	-2.70	0.008
Fragment size	0.22	0.03 – 0.41	80.5	2.30	0.02
Canopy cover	-0.16	-0.33 – 0.00	78.2	-1.98	0.05

Vertical vegetation structure	0.003	-0.16 – 0.16	88.4	0.04	1.0
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The results Mean H:L ratio was 0.98 ± 0.68 (range 0.17 – 3.5). The reference interval for the H:L ratio ranged from 0.2 (90% CI = 0.2 – 0.3) to 2.8 (90% CI = 2.4 – 3.5). The results showed that Greenbuls had higher H:L ratios in larger fragments than in smaller fragments. There was also a marginally significant ($p = 0.05$) relationship between H:L ratio and canopy cover with individuals breeding in areas with low canopy cover having higher H:L ratios than those breeding in areas with high canopy cover however, vertical vegetation structure was not significantly related to H:L ratio. Males had lower H:L ratios than females and H:L ratios differed between years

Relationship between fragment size and the H:L ratio

The results on the relationship between fragment size and the H: L ratio indicate that there was a positive relationship between fragment size and the H:L ratio in Placid Greenbuls. In larger fragments greenbuls had larger H:L ratios than in smaller fragments

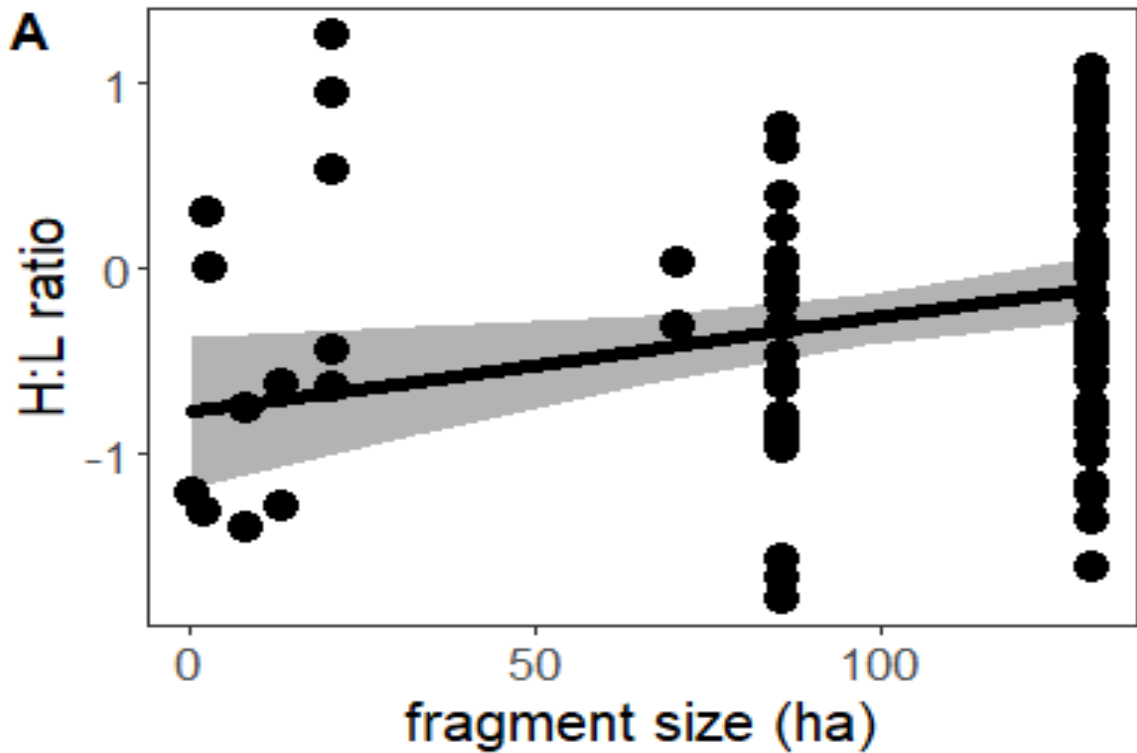


Fig 2. Relationship between H:L ratio and forest fragment size

The graph above shows that there is a positive relationship between fragment size and the H:L ratio, indicating that Placid Greenbuls in larger fragments had larger H:L ratios than in smaller fragments. It contradicts the common assumption that larger habitat fragments reduce physiological stress. This could be due to other factors such as increased competition and predation.

Relationship between H:L ratio and canopy cover

The graph below shows that Greenbuls had lower H:L ratios when canopy cover was higher

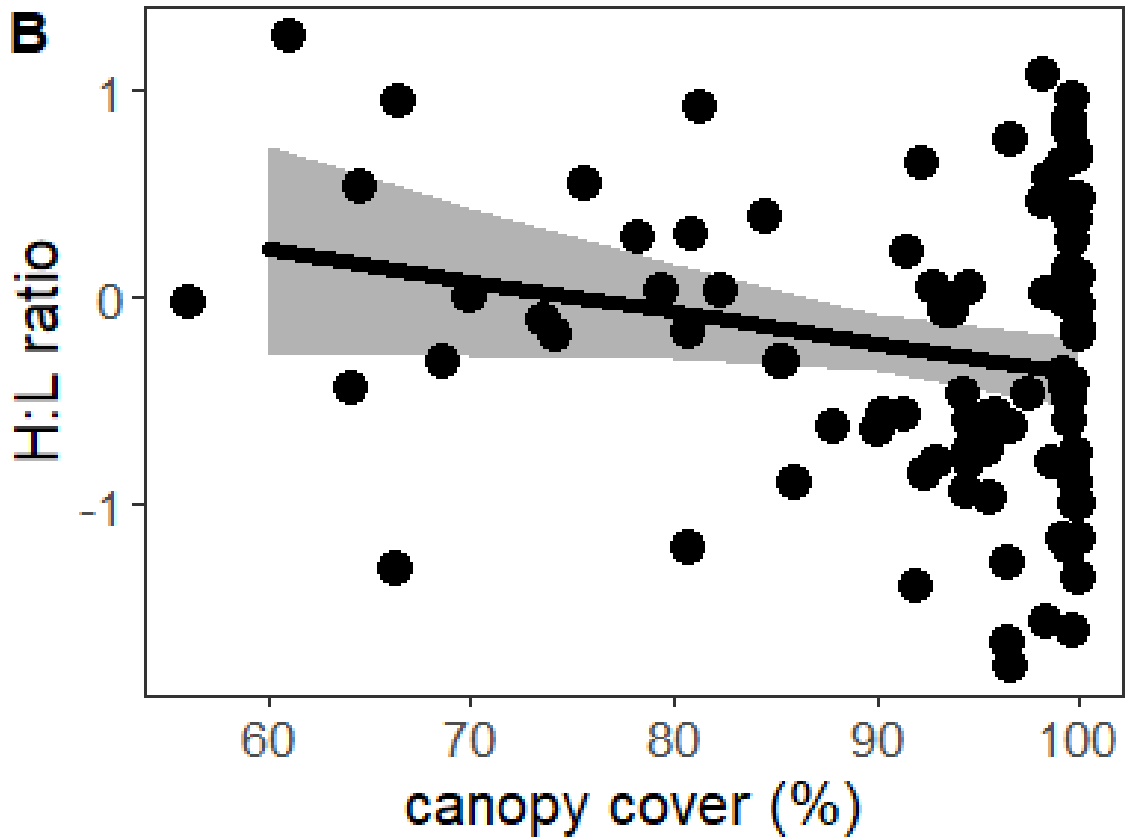


Fig 3. Relationship between H:L ratio and canopy cover

The graph above shows the relationship between canopy cover and H: L in Placid Greenbuls. It shows that H: L ratio of individuals in areas of higher canopy cover generally exhibiting lower H:L ratios denser habitats with more canopy cover may provide a less stressful environment for Placid Greenbul

Reduced canopy continuity imposes direct challenges, likely through increased foraging costs when individuals are forced to cross gaps or travel further between feeding patches. In contrast, vertical vegetation heterogeneity was not associated with H:L ratios, indicating that the presence or absence of dense canopy cover, rather than overall vegetation layering, is the key proximate driver of stress in adult breeders.

Relationship between H:L ratio and vertical vegetation heterogeneity

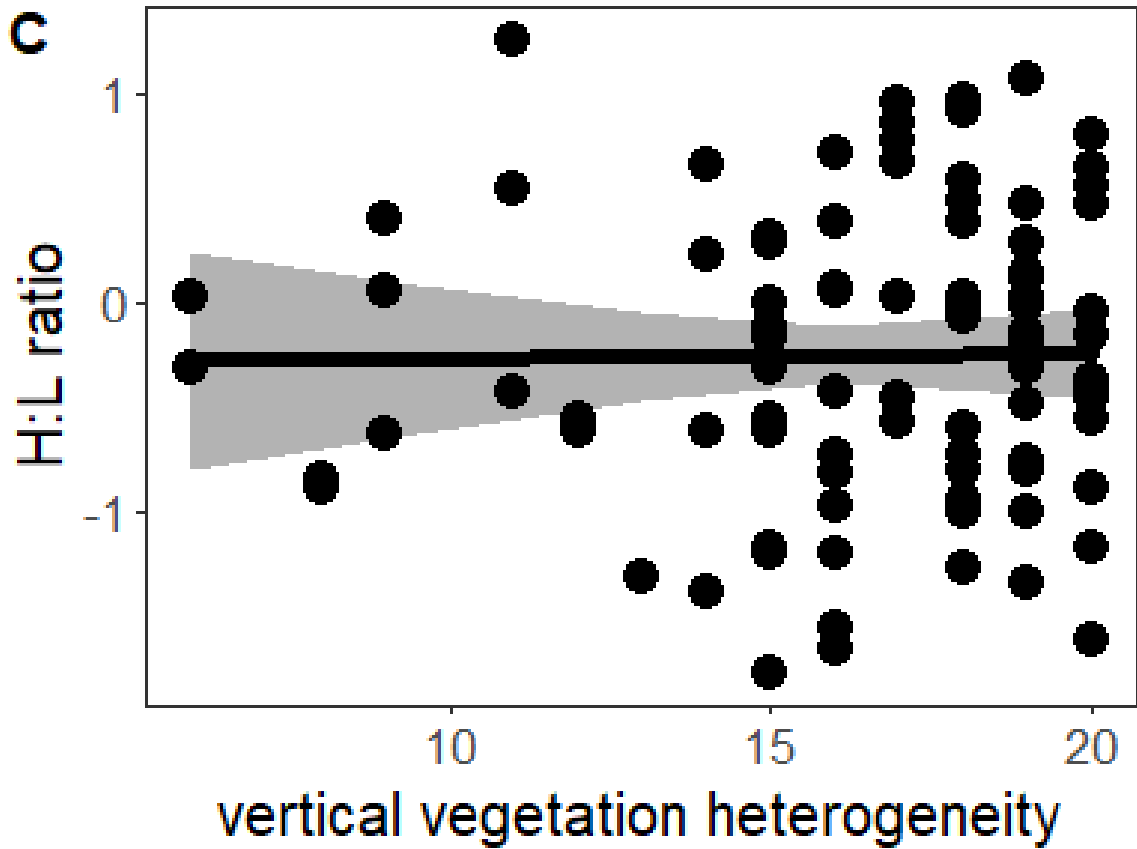


Fig 4 Relationship between H:L ratio and vertical vegetation heterogeneity

Vertical vegetation structure showed no relationship with the H:L ratio. This aligns with earlier research on corticosterone levels, which also found no correlation between vertical vegetation structure and H:L ratios. Consequently, adult greenbuls seem to be more affected by reduced canopy cover, as it compels them to travel longer distances (Kung'u et al., 2025), than by the structure of the vegetation itself. This may indicate flexibility on the part of greenbuls, which has been seen that they are foraging in all vegetation layers, avoiding only areas that completely lack canopy.

CHAPTER FIVE

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.0 Introduction

This chapter provides a summary and discussions of the findings of this study. This chapter also provides actionable recommendations that can guide future research, inform policy decisions, and conservation of the fragmented Taita Hills forests.

5.1 Discussions

Higher H:L ratio in the largest and most intact forest fragment paradox aligns with some previous studies that found poorer health indicators in continuous habitats, which were attributed to factors such as greater intraspecific competition for high quality territories or higher predator pressure in intact forest interiors. In the Taita Hills, nest predation rates have indeed been shown to be highest in the core of the largest fragment, providing a probable explanation for the elevated H:L ratios observed there. These results suggest that different stressors may dominate at different spatial scales: local canopy cover influencing energetic stress through food access, and fragment-level factors such as competition or predation shaping hematological stress responses.

This study has shown two patterns in physiological stress responses of breeding Placid Greenbuls, indicating that birds found in areas with reduced canopy cover showed higher H:L ratios, and unexpectedly, H:L ratios were highest in the largest and most intact forest fragment contradicting the original prediction that smaller, degraded fragments impose greater physiological stress.

Findings of this study are linking habitat quality to physiological stress, showing that habitat fragmentation and degradation cannot be reduced to a simple relationship between fragment size and stress but rather involve scale-dependent mechanisms and multiple stressor types.

Contrary to the predictions and previous studies, which have shown that the H:L ratio increases with decreasing habitat quality or size, H:L ratios in this study were highest in the large and intact fragment. In line with the predictions greenbuls breeding in territories with low canopy cover had higher H:L ratios than those breeding in territories with a dense canopy cover.

The breeding greenbuls have higher H:L ratios in individuals from territories with low canopy cover. These results indicate that greenbuls encounter more challenging conditions when breeding in territories with reduced canopy cover. A reason may be that greenbuls are reluctant to use canopy gaps and thus have to travel longer distances to foraging patches when canopy cover is low. Similar to the previous study on corticosterone levels, vertical vegetation structure was not correlated with H:L ratios. Thus, adult greenbuls seem to be more affected by lack of canopy cover than by vegetation structure per se.

Forest fragmentation also affects predator communities and thus can affect nest predation rates, although the direction of change may depend on a variety of landscape factors. For example, some studies in tropical areas found higher nest predation rates in the interior of fragmented habitats indicating that forest-dependent nest predators favour the interior of (bigger) forest fragments, because of less human disturbance and better habitat quality. However, this makes individuals nesting in the centre, especially in large habitat fragments, more susceptible to nest failures. This is maybe the case in the Taita Hills as well, where

nest predation on greenbuls was found to be highest in the centre of the largest, most intact fragment as Ngangao. The presence of predators has been shown to lead to changes in H:L ratio, although the direction of change is not consistent across

5.2 Summary of the study

This study has shown a complex, dependent relationship between habitat quality and physiological stress in Placid Greenbuls during breeding. There was a positive relationship between fragment size and H:L ratio indicating that birds in larger forest fragments exhibiting higher stress levels, this contradicted the expectation that larger, intact habitats reduce stress. This could likely be due to increased competition and predation pressures in the larger forest fragments.

Canopy cover showed a strong negative relationship with H:L ratios, indicating that denser canopy continuity provides a less stressful environment by lowering foraging costs and reducing exposure to risks when crossing habitat gaps.

5.3 Conclusions

Habitat degradation, particularly the reduction in canopy cover, appears to create challenging conditions for greenbuls during breeding, as reflected in elevated corticosterone levels and H:L ratios. However, higher H:L ratios in the largest, most intact forest fragment suggest that, despite higher potential physiological stress, intact ecosystems may offer greater overall ecological value. These areas likely provide more complete trophic networks, which support higher biodiversity, but may also create stressors such as increased competition for high-quality territories, parasitism, and predation. Our results suggest that habitat fragmentation, while potentially relaxing some stressors (such

as reduced competition in smaller fragments), also introduces complex trade-offs. The higher H:L ratios observed in the most intact fragment may reflect the physiological and immunological responses to these multifaceted challenges. These findings, together with previous data on greenbul movement behaviour and physiology, highlight the need for conservation strategies that balance both habitat connectivity and quality within forest fragments. Thus, effective conservation efforts in the Taita Hills should focus not only on maintaining connectivity between forest fragments (, but also on ensuring the preservation of forest quality, which supports the full range of ecological functions and species interactions.

5.4 Recommendations

From this study, it is recommended that future studies should be conducted to investigate the physiological responses of forest birds to habitat fragmentation including multiple indicators of stress in birds, such as both H:L ratios and corticosterone levels. Also recommend that future studies should be conducted to examine the role of landscape-level features and fragment-specific conditions, such as predator abundance, competition, and human disturbance. The higher H:L ratios observed in the largest fragment, Ngangao, may be explained by factors like increased nest predation or higher competition for territories stressors that do not necessarily align with traditional metrics of habitat quality. Incorporating data on predator presence, territorial density, and bird movement or behavior could help identify the mechanisms behind physiological stress responses. Longitudinal or multi-seasonal studies would also help account for temporal variability in stress and provide a clearer picture of how greenbuls adapt or fail to adapt to fragmented and degraded landscapes.

This study suggests that these indicators may reflect different types of environmental stressors and are not always directly correlated. Incorporating additional stress markers or behavioral indicators could provide a more nuanced understanding of how greenbuls and similar species respond to changes in their environment. Moreover, the unexpected finding of higher H:L ratios in the largest forest fragment suggests, ecological assumptions based solely on habitat size or integrity may overlook other influential factors such as predation pressure or intra-specific competition.

Future studies should be conducted including data on offspring condition, particularly nestlings, as previous studies have indicated that young birds are often more sensitive to habitat quality than adults. Including nestling H:L ratios and growth measures would help clarify how environmental stress impacts reproductive success and long-term population viability. Additionally, studies should aim for more balanced sampling across habitat types, especially in small fragments and areas with reduced canopy cover, to improve the robustness and generalizability of the findings.

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APPENDICES

Research permit

This study was conducted under the following research permit as it was a component of the project on Behavioural, physiological and fitness consequences of habitat fragmentation and degradation in an East African cloud forest bird species



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